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**EUROPEAN PATENT APPLICATION**

⑳ Application number: 84107498.2

㉔ Date of filing: 28.06.84

RECEIVED <sup>⑤1</sup> Int. Cl.<sup>4</sup>: C 07 K 13/00  
C 12 N 15/00, C 12 P 21/00  
C 07 H 21/04, A 61 K 45/02  
FEB 25 1985  
PATENT LAW

③0 Priority: 01.07.83 GB 8317880

④3 Date of publication of application:  
23.01.85 Bulletin 85/4

⑤4 Designated Contracting States:  
BE CH DE FR GB IT LI NL SE

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⑤4 Modified (1-56) beta interferons.

⑤7 Modified beta interferons containing amino acid substitutions in the beta interferon amino acids 1 to 56 are described. These modified beta interferons exhibit changes in the antiviral, cell growth regulatory or immunomodulatory activities when compared with unmodified beta interferon.

EP 0 131 816 A1

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**PARTIAL EUROPEAN SEARCH REPORT**  
which under Rule 45 of the European Patent Convention  
shall be considered, for the purposes of subsequent  
proceedings, as the European search report

**0131816**

Application number

DOCUMENTS CONSIDERED TO BE RELEVANT			EP 84107498.2
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. $\times$ 4)
A, P, D	WO 83/02 461 (CETUS) * Claims 1-6, 9, 12-16, 19-23, 26-30, 33, 34-38, 41-44 * --	1-62, 64	C 07 K 13/00 C 12 N 15/00 C 12 P 21/00 C 07 H 21/04
A, D	GB - A - 2 090 258 (GENENTECH) * Claims 1, 8, 10, 12, 18 * --	1, 33, 34, 46, 48, 50	A 61 K 45/02
P, A, D	US - A - 4 414 150 (GOEDDEL) * Claim 1 * ----	1, 50	
			TECHNICAL FIELDS SEARCHED (Int. Cl. $\times$ 4)
			C 07 K C 12 N C 12 P C 07 H A 61 K
<b>INCOMPLETE SEARCH</b>			
<p>The Search Division considers that the present European patent application does not comply with the provisions of the European Patent Convention to such an extent that it is not possible to carry out a meaningful search into the state of the art on the basis of some of the claims.</p> <p>Claims searched completely: 1-62 Claims searched incompletely: 64 Claims not searched: 63, 65 Reason for the limitation of the search:</p> <p>method for treatment of the human or animal body by therapy (Article 52(4))</p> <p style="text-align: center;">114</p>			
Place of search VIENNA		Date of completion of the search 19-09-1984	Examiner FARNIOK
<b>CATEGORY OF CITED DOCUMENTS</b>			
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document	

SPECIFICATION

## BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention describes the use of recombinant DNA technology for the design and synthesis of novel modified interferons. More specifically the invention relates to interferons not known in nature, which are intended for use in viral and neoplastic diseases, and immunosuppressed and immunodeficient conditions.

2. Description of the Prior Art

The interferons are a class of proteins that occur in vertebrates and act as biological regulators of cell function which include increasing resistance to pathogens, limiting cell growth and modulating the immune system. The most studied property of the interferons is their ability to convert cells into an "antiviral state" during which they are more resistant to virus replication (Lengyel, Annual Review of Biochemistry, 51, 251, 1982). In addition to conferring antiviral resistance to target cells, interferon (IFNs) have antiproliferative (antigrowth) properties (Stewart, 1979, The Interferon System, Springer, Berlin). It has clearly been shown that interferons produced naturally act as antiviral and antiproliferative agents (Gresser et al, Biochim. Biophys. Acta, 516, 231, 1978; J. Exp. Med., 144, 1316, 1976).

The IFNs, by virtue of their antigenic, biological and physico-chemical properties, may be divided into three classes: type I, IFN- $\alpha$  ("leucocyte") and IFN- $\beta$  ("fibroblast"); and type II, IFN- $\gamma$  ("immune") (Stewart *et al*, Nature, 286, 110, 1980). Both genomic DNA cDNA clones of type I and type II IFNs have been isolated and sequenced, and the potential protein sequences deduced (e.g. Pestka, Arch. Biochem. Biophys., 221, 1, 1983). While in man only one IFN- $\beta$  and IFN- $\gamma$  gene are known, human IFN- $\alpha$  is specified by a multigene family comprising at least 20 genes. The classification of IFN- $\beta$  and IFN- $\alpha$  as type I interferons is in part determined by their significant degree of homology, >23% at the protein level (Taniguchi *et al*, Nature, 285, 547, 1980).

While the mechanism of action of interferons is not completely understood, certain physiological or enzymatic activities respond to the presence of the interferons. These activities include RNA and protein synthesis. Among the enzymes induced by interferons is (2'-5') (A)n synthetase generates 2'-5' linked oligonucleotides, and these in turn activate a latent endoribonuclease, RNase L, which cleaves single-stranded RNA, such as messenger RNA (mRNA) and ribosomal RNA (rRNA). Also induced by IFNs is a protein kinase that phosphorylates at least one peptide chain initiation factor and this inhibits protein synthesis (Lengyel. *ibid*, p 253) IFNs have been shown to be negative growth regulators for cells by regulation of the (2'-5') An synthetase activity (Creasey *et al*. Mol and Cell Biol.. 3, 780, 1983) IFN- $\beta$  was indirectly shown to be involved in

the normal regulation of the cell cycle in the absence of inducers through the use of anti-IFN- $\beta$  antibodies.

Similarly, IFNs have been shown to have a role in differentiation (Dolei et al. J Gen Virol . 46. 227. 1980) and in immunomodulation (Gresser. Cell Immunol . 34. 406 1977) Finally IFNs may alter the methylation pattern of mRNAs and alter the proportion of fatty acids in membrane phospholipids thereby changing the rigidity of cell membranes

These and other mechanism may respond to interferon-like molecules in varying degrees depending on the structure of the interferon-like polypeptide Preliminary evidence (UK Patent GB 2 090 258A) suggests that members of the multigene IFN- $\alpha$  family vary in the extent and specificity of their antiviral activity (Pestka. ibid ) For example combination of IFN- $\alpha$ A with IFN- $\alpha$ D resulted in "hybrid" genes which show antiviral properties that are distinct from either parent molecule (Weck et al Nuc. Acids Res 9. 6153 1981; De La Maza et al. J IFN Res . 3. 359. 1983; Fish et al, Biochem Biophys Res Commun . 112. 537 1983; Weck et al. Infect Immun . 35 660 1982) However hybrid human IFNs with significantly increased human cell activity/specificity have not yet been developed One Patent has been published describing IFN- $\beta$ / $\alpha$  hybrids (PCT/US83/00077) This patent describes three examples. none of which have significantly improved activity. The three examples were constructed using two naturally occurring restriction sites The resulting hybrid interferons were 1) alpha 1 (1-73)-beta (74-166); 2) beta (1-73)-alpha 1 (74-166); and 3) alpha 61A

(1-41)-beta (43-166) These three examples differ structurally from the examples of the present invention. These three examples were based upon the accidental location of two restriction sites and not upon the intentionally designed DNA and amino acid sequences of the present invention.

It is envisioned that a modified interferon will display a new advantageous phenotype. The design and synthesis of new interferon-like polypeptides composed of portions of IFN- $\beta$  and other amino acid sequences is advantageous for the following reasons:

1. New IFNs can be created which show a greater antiproliferative to antiviral activity (and vice versa) resulting from the selective activation of only some of the normal interferon-induced biochemical pathways.
2. The affinity of hybrid or modified IFNs for cell surface receptors can differ from that of naturally occurring interferons. This will allow selective or differential targeting of interferons to a particular cell type or increased affinity for the receptor - leading to increased potency against a particular virus disease or malignancy.
3. It will be possible to design novel IFNs which have an increased therapeutic index thus excluding some of the undesirable side effects of natural IFNs which limit their use (Powledge, TM Biotechnology, 2, 214, March 1984).

4. Novel IPNs can include in the design structures which allow increased stability to proteolytic breakdown during microbial synthesis.
5. Novel IPNs can be designed to increase their solubility or stability in vivo, and prevent non-specific hydrophobic interactions with cells and tissues.
6. Novel IPNs can be designed which are more readily recovered from the microbial supernatant or extract, and more easily purified.

Additional Relevant Patent Applications

UK No. GB2 116 566<sup>a</sup> - Animal interferons and processes for their production

US No 4 414 150 - Hybrid human leukocyte interferons

UK No. GB 2 068 970<sup>a</sup> - Recombinant DNA technique for the Preparation of a protein resembling human interferon

SUMMARY OF THE INVENTION

Recombinant DNA technologies were successfully applied to produce modified beta interferon-like polypeptides nucleic acids (either DNA or RNA) which code for these modified beta interferons plasmids containing the DNA coding for the modified beta interferons and procedures for the synthesis of these modified beta interferons Each of the amino acids 1-56 of human beta interferon may individually be replaced by any other amino acid This replacement may be accomplished in groups of three to fifty-six amino acids

One preferred embodiment is the replacement of each amino acid from 2 to 7 and 9 to 56 of human beta interferon by another amino acid. Another preferred embodiment is the replacement of each beta interferon amino acid from 9 to 56 by four to forty-seven other amino acids. The beta interferon amino acids 2 to 7 and 9 to 56 may be replaced by corresponding sequential human alpha interferon amino acids. Among the alpha interferons are alpha 1, alpha 2 and alpha H. The alpha and beta interferons from any mammal may be used, including but not limited to humans or other primates, horses, cattle, sheep, rabbits, rats and mice. In one embodiment of the invention, the cysteine 17 or methionine 31 in human beta interferon may optionally be replaced by serine 17 (or leucine 17) and/or lysine 31. In some examples, e.g., IFNX410 (Chart 3d), the cysteine or leucine or serine at position 17 is renumbered as position 16 because the inserted amino acids upstream of position 17 contain one less amino acid. Yet another embodiment of the invention discloses the use of the modified beta interferons where in one or more of the antiviral, cell growth regulatory or immunomodulatory activities is substantially changed from that of the unmodified beta interferon. Particularly preferred embodiments are the amino acid sequences of IFNX402, 403, 404, 406, 407, 408, 409, 410, 415, 419 and 420. Yet another preferred embodiment of the invention is DNA or RNA sequences which code for the synthesis of IFNX402, 403, 404, 406, 407, 408, 409, 410, 415, 419 or 420. Yet another embodiment of the invention is a pharmaceutical composition containing an effective amount of IFNX402, 403, 404, 406, 407, 408, 409, 410, 415, 419, 420. A final embodiment of the invention is



the use of pharmaceutical compositions containing the modified beta interferons in a method of treating viral infections. regulating cell growth or regulating the immune system.

It was reported that the change of amino acid residue 17 from cysteine to serine markedly increased the specific antiviral activity of IFN- $\beta$  produced in E.coli (TNO Interferon meeting Rotterdam April 1983) In the present invention this result is not confirmed. and the alterations in biological activity demonstrated by some of the novel IFNs (shown in Tables 1-16) are therefore not due to the Ser<sup>17</sup>. Other amino acids may be present at residue 17 (or residue 16 e.g. Chart 3d). such as cysteine leucine or alanine In the present disclosure. the amino acid at residue 17 is either cysteine. serine or leucine.

Novel, modified IFNs with increased biological activity are disclosed in the present invention which may be more effective in the treatment of viral or neoplastic diseases or immunosuppressed or immunodeficient conditions. Novel. modified IFNs are disclosed which have substantially lost one or other of the activities measurable in vitro (e.g. antiviral. antiproliferative or immunomodulatory).

An increased target cell specificity or an increase in IFN activity can result in an improved therapeutic index This should exclude some of the side effects caused by the use in humans of naturally occurring IFNs.

This invention relates to the production in sufficient amounts of novel. highly active. and/or highly specific interferon-like molecules suitable for the prophylactic or therapeutic treatment of humans - notably for viral infections malignancies and immunosuppressed or immunodeficient conditions

Brief Description of the charts and tables

- Figure 1 shows the Sternberg-Cohen 3D model of  $\alpha_1$  and  $\beta$  interferons.
- Chart 2 (a to g) shows the ligated oligonucleotides used in the construction of the novel modified IFN genes
- Chart 3 (a to j) shows the complete nucleotide sequences of the novel. modified IFN genes and the encoded amino acid sequences
- Chart 4 shows the nucleotide sequence of the trp promoter used to initiate transcription of the novel. modified IFN genes
- Table 1 compares expression. antiviral and antiproliferative activities in bacterial extracts for some novel. modified IFNs
- Table 2 compares antiviral activities of IFN- $\beta$ , IFNX805 and IFNX415 in 3 different cell lines.
- Table 3 compares antiproliferative activities of IFNX805 and IFNX415 in 3 different cell lines.

Table 4 compares the ability of purified IFN- $\beta$ , IFN $\alpha$ 805 and IFN $\alpha$ 415 to stimulate Antibody-Dependent Cellular Cytotoxicity (ADCC).

## DESCRIPTION OF THE PREFERRED EMBODIMENTS

### Introduction

The IFN- $\beta$  gene is a unique gene but shows some significant homologies to the multigenic IFN- $\alpha$  family (Rubinstein. Biochim Biophys. Acta, 695. 5. 1982) Sternberg and Cohen (Int. J Biol Macromol.. 4. 137. 1982) have proposed a similar secondary structure for IFN- $\beta$  and IFN- $\alpha$ . Structure prediction studies suggest four  $\alpha$ -helices which can be "packed" into a right-handed bundle (Figure 1) similar to that observed in several unrelated protein structures as determined by X-ray crystallography. The design of some of the modified interferons described herein is derived from our interpretation of the Sternberg/Cohen model. Since IFN $\beta$  and  $\alpha$  are believed to bind to the same receptor at the cell surface it is possible to introduce variability into IFN- $\beta$  by replacing specific areas with IFN- $\alpha$  segments or any other amino acid sequence. The construction of these interferons has resulted in novel, hybrid interferons with altered biological properties. All these interferons were active to some degree suggesting a large measure of variability in the nature of the inserted amino acid sequence which would give rise to an active molecule.

In this invention each amino acid in the 1 to 56 region can be replaced by any other naturally occurring amino acid. The naturally occurring amino acids and their nomenclature are: alanine (Ala or A); valine (Val or V); leucine (Leu or L); isoleucine (Ile or I); proline (Pro or P); phenylalanine (Phe or F); tryptophan (Trp or W); methionine (Met or M); glycine (Gly or G); serine (Ser or S); threonine (Thr or T); cysteine (Cys or C); tyrosine (Tyr or Y); asparagine (Asn or N); glutamine (Glu or Q); aspartic acid (Asp or D); glutamic acid (Glu or E); lysine (Lys or K); arginine (Arg or R); and histidine (His or H).

Accordingly the field of the present invention is the design, synthesis and characterization of interferon-like molecules related to IFN- $\beta$  which may have IFN- $\beta$  amino acid sequences replaced with any other amino acid sequence, unrelated protein sequence, or sequences similar to those of IFN- $\alpha$ s, - $\beta$  or - $\gamma$  found in mammals and other vertebrates.

Though binding of hybrid IFN- $\kappa$ 's ( $\kappa_1$  and  $\kappa_2$  in Streuli et al. Proc Natl. Acad. Sci USA 78, 2848, 1981), an attempt was made to analyse the number and nature of idiotypes involved in the receptor binding site of IFN- $\kappa$ 's. Two sites were proposed as constituting the binding site, one in the amino-terminal half and the other in the carboxy-terminal half of IFN- $\kappa$ . The two major regions of partial homology between IFN- $\kappa$ 's and IFN- $\beta$  occur between amino acid residues 28-80 and 115-151 which may well correspond to the above mentioned idiotypes. Evidence that the 28-80 region may be important in receptor binding comes

from the finding that polyclonal antibodies raised against a synthetic peptide composed of IFN- $\alpha_2$  amino acids 24-81. bind to IFN- $\alpha_2$  and prevent it interacting with its cell receptor (Dreiding. TNO Interferon Meeting. Rotterdam 1983) The modified interferons of this invention, such as IFNX402 (IFN- $\beta$ ( $\beta$ (9-56))-( $\alpha_1$ (7-54))) display dramatically reduced human cell antiviral and natural killer cell activities relative to antiproliferative activity. Other examples of novel interferons derived from IFN- $\beta$  having altered amino acids between IFN- $\beta$  residues 1-56 were among those synthesized. IFN- $\beta$ ( $\beta$ (9-56)→ $\alpha_1$ (7-54))) denotes that amino acid residues 9-56 inclusive of IFN- $\beta$  are replaced by residues 7-54 of IFN- $\alpha_1$ . These examples illustrate the invention and are not intended to limit the scope of the invention in any way. Below are described techniques used in the design chemical synthesis and insertion of DNA fragments in the 1-56 region of the human IFN- $\beta$  gene The resultant novel, modified IFNs are hereafter described as group II IFNs. Decreased antiviral or increased antiproliferative activity are among the altered properties shown by some of the group II novel IFNs with amino acid replacements in the 1-56 region. The techniques described will be familiar to anyone skilled in the art (e.g. see also Molecular Cloning A Laboratory Manual, eds Maniatis et al, Cold Spring Harbor Laboratories).

#### Design of the synthetic gene fragments

The nucleotide sequences of each synthetic DNA fragment (Charts 2a-2e) were designed utilizing the following criteria:

1. Codon utilization (where it deviates from IFN- $\beta$  gene sequence) was optimized for expression in E.coli. Natural IFN- $\beta$  gene sequences were used as far as possible in order to obtain levels of expression of novel IFNs as high as that of IFN- $\beta$  from plasmid pGC10 (see Table 1). pGC10 (~4,440bp) expresses the natural IFN- $\beta$  gene at a high level and is identical to pl/24 (Searle Patent GB 2 068 970A, hereby incorporated by reference) except for the ribosome binding site sequence shown in Chart 4 and the deletion of the ~546bp PstIII-BamHI fragment.
2. Sequences which might anneal to each other in the assembly of the chemically synthesized fragment (Chart 2) were not included in the design (within the limits allowed by the redundancy in the genetic code)

#### Chemical Synthesis of Gene Fragments

Oligodeoxyribonucleotides were synthesized by the phosphoramidite method (M.H. Caruthers in "chemical and Enzymatic synthesis of Gene Fragments". ed. H.G. Gasen and A. Lang Verlag Chemie, 1982. p 71) on controlled pore glass (H. Koster et al. Tetrahedron. 40. 103 1984). Fully protected 2'-deoxyribonucleotide 3'-phosphoramidites were synthesized from the protected deoxyribonucleotide and chloro-N,N-(diisopropylamino)methoxyphosphine (L.J. McBride and M.H. Caruthers Tetrahedron Lett. 24. 245. 1983 and S.A. Adams et al. J. Amer. Chem. Soc., 105. 661. 1983) Controlled pore glass supports were synthesized as described

'P. Chow et al. Nuc Acids Res . 1981 9. 2807) giving 30-50 $\mu$ mol deoxynucleoside per gram.

The functionalised controlled pore glass (50mg) was treated in a sintered glass funnel at ambient temperature sequentially with:

1. dichloromethane (3ml 10s)
2. 3% (V/v) dichloroacetic acid in dichloromethane (2ml 120s)
3. dichloromethane (3ml 10s)
4. anhydrous acetonitrile (3ml 10s)
5. phosphoramidite monomer (0.06M)/tetrazole (0.23M) in anhydrous acetonitrile (1ml. 120s)
6. acetonitrile (3ml. 10s)
7. dimethylaminopyridine (0.07M) in acetic anhydride/2,6-lutidine/acetonitrile (1/2/6<sup>V</sup>/v) (1ml. 60s)
8. acetonitrile (3ml 10s)
9. iodine (0.2M) in 2,6-lutidine/tetrahydrofuran/water (1/2/2<sup>V</sup>/v) (1ml. 30s)
10. acetonitrile (3ml. 10s)

The cycle was repeated with the appropriate phosphoramidite monomer until the immunogenetic chain was complete. The coupling efficiency of each cycle was monitored by spectrophotometric assay of the liberated dimethoxytrityl alcohol in 10% (W/v) trichloroacetic acid/dichloromethane at 504nm. After completion of the synthesis the protecting groups were removed and the

oligomer cleaved from the support by sequential treatment with 3% (v/v) dichloroacetic acid/dichloromethane 9120s). thiophenol/triethylamine/dioxan (1/1/2<sup>v</sup>/v) (1h) and concentrated ammonia at 70°C (4h). The deprotected oligonucleotides were purified either by HPLC on a Partisil 10 SAX column using a gradient from 1M to 4M triethylammonium acetate pH4.9 at 50°C or by electrophoresis on a denaturing 15% polyacrylamide gel (pH8.3)

#### Ligation of Oligonucleotide Blocks

500 pmole aliquots of the oligonucleotides were phosphorylated with 1 unit of T<sub>4</sub> induced polynucleotide kinase in 20 µl of a solution containing 1000 Ci/pmole [<sup>32</sup>P]γ-ATP (2.5 Ci/mMole), 100 µM spermidine, 20mM DTT 10mM MgCl<sub>2</sub>, 50mM Tris-HCl (pH9.0) and 0.1mM EDTA for 60 minutes at 37°C. The mixtures were then lyophilized and each oligonucleotide purified in a denaturing 15% polyacrylamide gel (pH8.3). After elution from the gel, the recovery was determined by counting the radioactivity.

Blocks (length 30-50 bases) were assembled by combining 25 pmole of each phosphorylated component with equimolar amounts of the unphosphorylated oligomers from the complementary strand. The mixtures were lyophilized and then taken up in 15 µl water and 2 µl 10 x ligase buffer (500mM Tris-HCl pH7.6, 100mM MgCl<sub>2</sub>). The blocks were annealed at 100°C for 2 minutes then slowly cooled to room temperature (20°C). 2 µl 200mM DTT and 0.5 µl 10mM ATP were added to give final concentrations of 20mM DTT and 250 µM ATP in 20 µl. 1.25 units of T<sub>4</sub> DNA ligase were also added. After 18 hours



at 20°C. the products were purified in a 15% polyacrylamide gel under denaturing conditions

Two duplex blocks were then constructed from the single-stranded pieces. (These were 150 base pairs and 75 base pairs). 1.5 pmole of each block were taken and the mixtures lyophilized. Annealing was carried out in 15 µl water and 2 µl 10 x ligase buffer at 100°C for 2 minutes then slowly cooled to 10°C. 2 µl 200mM DTT 0.5 µl 10mM ATP and 1.25 units T4 DNA ligase were added. The reaction was left at 10°C for 18 hours. The products were then purified in a 10% native polyacrylamide gel.

The final product was assembled by combining 0.4 pmole of the two duplexes. The mixture was lyophilized and then taken up in 15 µl water and 2 µl 10 x ligase buffer. It was annealed at 50°C for 2 minutes and then slowly cooled to 10°C. 2 µl 200mM DTT 0.5 µl 10mM ATP and 1.25 units ligase were then added and the reaction left at 10°C for 18 hours. The final product was purified in a 5% native polyacrylamide gel. After elution and ethanol precipitation the product was taken up in 10 µl water. 0.5 µl were removed for counting to calculate the recovery. 2 µl 10 x ligase buffer, 2 µl 200mM DTT, 2 µl 1mM spermidine, 1 µl 10mM ATP, 3 µl water and 0.5 units kinase were added to the rest (total volume 20 µl). The reaction was left at 37°C for 1 hour and stopped by heating at 90°C for 2 minutes. The final product was ethanol precipitated.

Construction of plasmids expressing novel modified  
interferons.

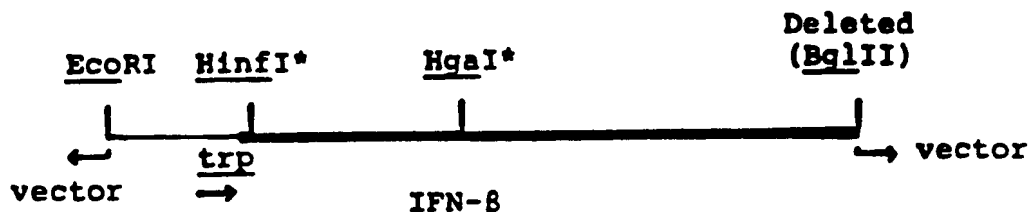
This section lists and identifies the vectors employed in the cloning of the synthetic DNA fragments (Chart 2) into the IFN- $\beta$  coding region. the restriction enzyme sites\* used for the insertion, and the rationale for the construction. The positions of these sites\* are shown relative to the complete coding nucleotide sequences of the group II novel IFN genes (Chart 3) The IFN- $\beta$  (or novel IFN) coding region is shown as a heavy line and would be translated from left to right. The vector sequences between the BglII (or BamHI) site and the EcoRI site are the same as those in pAT153 (equivalent to pBR322 with a 705bp HaeII fragment deleted - nucleotides 1,646-2,351 on the map). A vector denoted by "m" instead of "p" refers to M13 mp8 sequences between the EcoRI and BamHI sites. The E. coli trp promoter (Chart 4) lies between the EcoRI site and Clal site (or equivalent position in IFNX407, X408 and X409).

Example 1

IFNX407 IFN- $\beta$  [ $\beta^{9-56} \rightarrow \alpha_1^{7-54}$ ]

This is IFNX402 with a change from Cys<sup>17</sup> to Leu<sup>17</sup> and was designed to determine the effect of residue 17 on antiviral and antiproliferative activity. Starting vector: pMN39-1. pMN39-1 is identical to pl/24 (UK Patent - Application GB 2 068 970A) except that the 546bp BglII-BamHI fragment is deleted.

PMN39-1



a synthetic oligonucleotide (Chart 2a) was inserted between the HinfI\* and HgaI\* sites to give the nucleotide sequence shown in Figure 3a. INFX407 is expressed from plasmid pJA29.

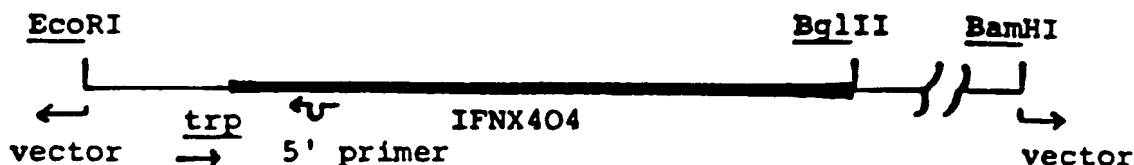
Example 2

INFX408 IFN-β<sub>1</sub><sup>44-56</sup> → α<sub>1</sub><sup>42-54</sup> [Cys<sup>17</sup> → Ser]

This is derived from INFX404 and was designed to determine the effect of the change of residue 17 from Cys to Ser (IFN-β with Ser<sup>17</sup> was first disclosed by Cetus Corp., TNO Interferon Meeting. Rotterdam. April 1983).

Starting vector: pXX404. pXX404 is similar to p1/24 (UK Patent Filing Application GB 2 068 970A), except that the IFN-β amino acid residues 44-56 are replaced by IFN-α<sub>1</sub> residues 42-54.

pXX404



The Cys<sup>17</sup>→Ser change to pXX404 was accomplished by oligonucleotide-directed (or site-directed) mutagenesis (Zoller and Smith, Nucl. Acids Res . 10. 6487. 1982). An EcoRI-BamHI (~1,176bp) fragment was subcloned in M13 mp8 for mutagenesis using the primer 5'-CTGACTCTGAAAATTG-3' to give M13 recombinant, mJA8. Clones with the codon 17 sequence 5'-AGT-3' (Ser) were isolated (mJA9) and an EcoRI-BglII fragment subcloned in the EcoRI-BamHI vector fragment of pXX404 to give IFNX408 expression plasmid pJA27 (Chart 3b).

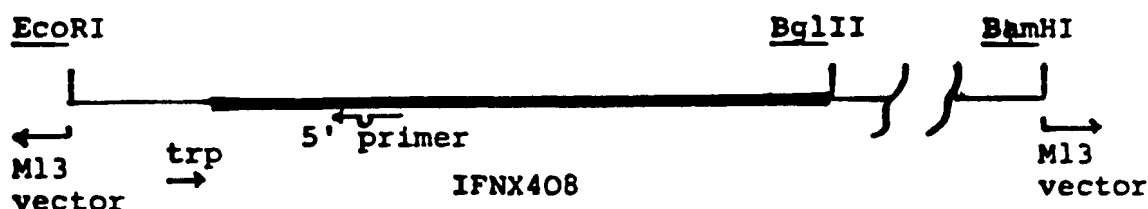
Example 3

IFNX409 IFN-β[β<sup>42-56</sup>→<sub>1</sub><sup>40-54</sup>][Cys<sup>17</sup>→Ser]

IFNX409 is an analogue of IFNX408 (and IFNX404) and was designed to replace the Glu at amino acid residue 42 with Gln to test the effect of changing the predicted secondary structure, since the Glu-Glu-Glu sequence (at residues 42-44) was considered unfavourable.

Starting vector: mJA9 'see above'. This phage M13 vector contains the entire coding sequence of IFNX408 on an EcoRI-BamHI fragment.

mJA9



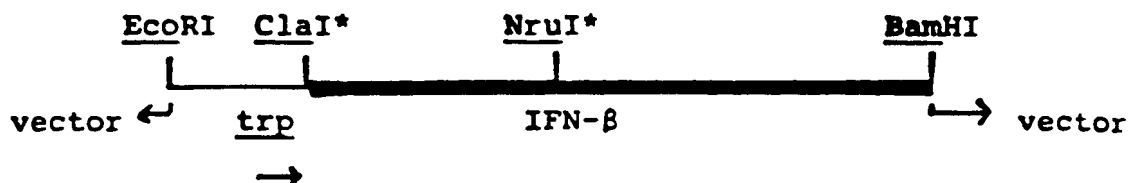
The Glu<sup>42</sup> → Gln change to mJA9 was accomplished by oligonucleotide-directed mutagenesis as above using the primer 5'-AAACTCTTCTTGAGGGATGTC-3'. with the modification described by Norris et al. Nucl. Acids Res., 11. 5103 1983. Clones with the codon 42 sequence 5'-CAA-3' (Gln) were isolated and an EcoRI-BglII 620bp fragment subcloned in the EcoRI-BamHI vector fragment of pXX404 to give pJA31. the plasmid expressing IFNX409 (Chart 3c).

Example 4

IFNX410 IFN- $\beta$   $1^2-7 \rightarrow \alpha_2^{1-5}$   $1 \beta^{9-56} \rightarrow \alpha_1^{7-54}$

This modified, novel IFN was constructed to investigate the additive, synergistic or other effect of combining in one molecule sequences from two different IFN- $\alpha$ 's. IFNX410 is related to IFNX402.

Starting vector: pMN47. This vector contains an entirely synthetic IFN- $\beta$  gene (Chart 3j) inserted between the ClaI and BamHI sites of pl/24C (pl/24C is identical to pl/24 except for the underlined sequences in Chart 4).

PMN47

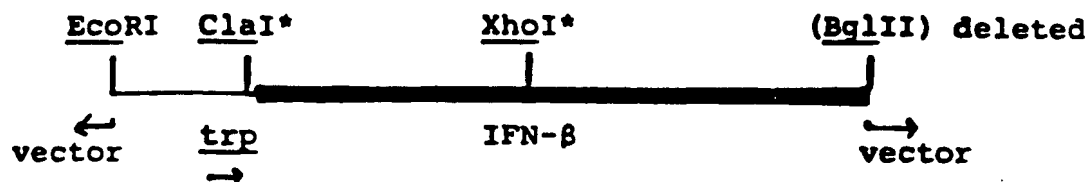
A synthetic oligonucleotide (Chart 2b) was inserted between the ClaI\* and NruI\* sites to give the nucleotide sequence shown in Chart 3d. IFNX410 is expressed from plasmid pAS213.

Example 5

IFNX415 IFN-β [ $\beta^{28-46} \rightarrow \chi_1^{28-46}$ ] [Cys<sup>17</sup> → Ser] [Met<sup>31</sup> → Lys]

This novel, modified IFN was designed to test the generality and extent of substitutions in the 9-56 region of IFN-β causing decreased antiviral activity, and to enhance or depress other IFN activities.

Starting vector: pAP4. pAP4 expresses IFN-β and is identical to pGC10 except that the serines at amino acid residues 74 and 75 are coded by TCC and TCG, respectively. These serine codons were changed from TCA and TCT in order to introduce an unique XhoI site to facilitate the insertion of synthetic DNA. This was accomplished by oligonucleotide-directed mutagenesis (Zoller and Smith, Nucl. Acids Res . 10. 6487. 1982) using the mismatch primer: 5'-CAGTGCTCGAGGAATCTTGTC-3'.

pAP4

A synthetic oligonucleotide (Chart 2c) was inserted between the ClaI\* and XhoI\* sites of pAP4 to give the nucleotide sequence shown in Chart 3e. IFNX415 is expressed from plasmid pAP7.

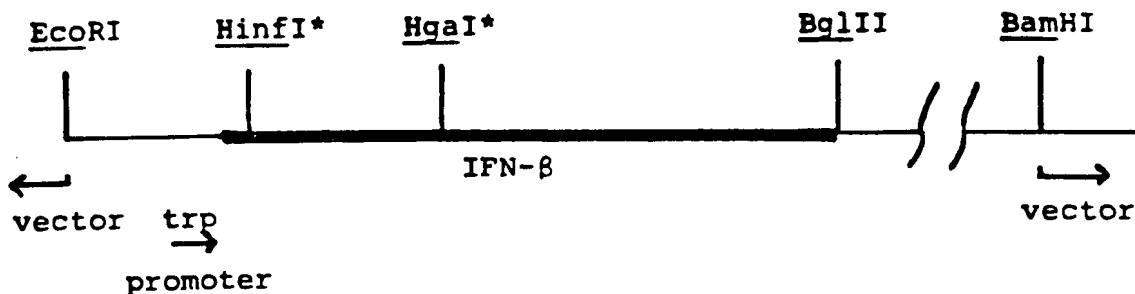
Example 6

IFNX402 IFN- $\beta$   $\beta^{9-56} \Rightarrow \alpha_1^{7-54}$  || Leu<sup>17</sup>  $\rightarrow$  Cys<sup>17</sup> |

This novel, modified IFN was designed to examine the effect of substituting a region from IFN- $\alpha_1$  into IFN- $\beta$  on relative antiviral, antiproliferative and immunostimulating activities.

Starting vector: pl/24, as above

It contains the mature, natural human IFN- $\beta$  gene which is expressed under trp promoter control.

pl/24

A synthetic oligonucleotide (Chart 2d) was inserted between the *HinfI*\* and *HgaI*\* sites of pl/24 to give the nucleotide sequence shown in Chart 3f. IFNX402 is expressed from plasmid pXX402. The ~546bp *BglII*-*BamHI* fragment is then deleted to obtain high level expression.

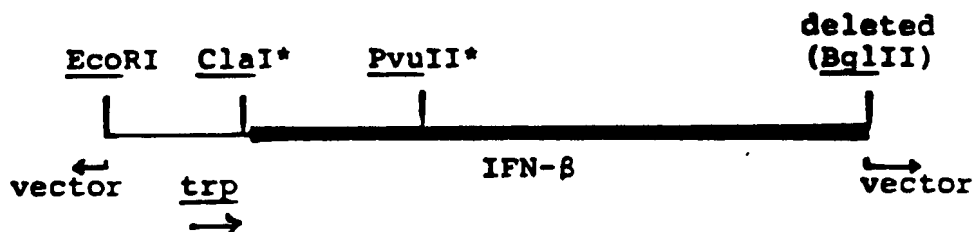
Example 7

IFNX419 IFN- $\beta$   $\beta^{9-42} \rightarrow \beta^{7-40}$

This novel, modified IFN was designed to test the generality and extent of substitutions in the 9-56 region of IFN- $\beta$  causing decreased antiviral activity and to enhance other IFN activities. The positioning of the substitution reflects the difference between IFNX402 and IFNX404.

Starting vector: pGC10



pGC10

A synthetic oligonucleotide (Chart 2e) was ligated between the ClaI\* and PvuII\* sites of pGC10 to give the nucleotide sequence shown in Chart 3g. IFNX419 is expressed from plasmid pAP6.

Example\_8

IFNX420 IFN- $\beta$  [ $\beta^{21-42} \rightarrow \alpha_1^{19-40}$ ] [Cys<sup>17</sup>  $\rightarrow$  Ser]

The rationale for construction was the same as for IFNX415; the changed amino acid sequence was a subset of the changed amino acid sequence in IFNX419 ( $\alpha_1^{7-40}$ ). A synthetic oligonucleotide (Chart 2f) was ligated between the ClaI\* and PvuII\* sites of pMN47 to give the nucleotide sequence shown in Chart 3h. IFNX420 is expressed from plasmid pNW25.

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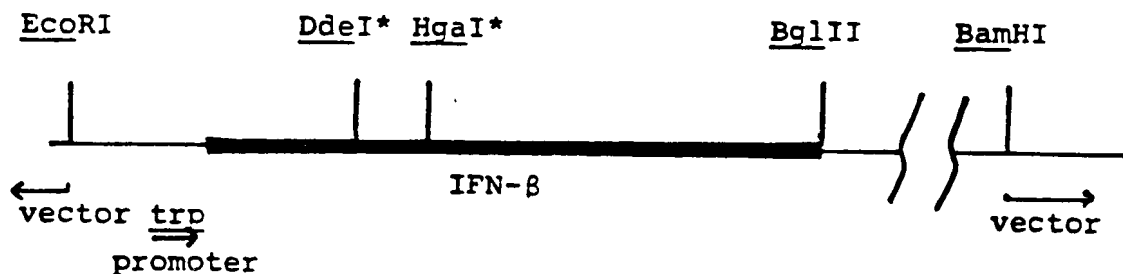
Example 9

IFNX404 IFN- $\beta$  |  $\beta^{44-56} \rightarrow \alpha_1^{42-54}$

This novel, modified IFN was designed to examine the effect of substituting a region from IFN- $\alpha_1$  into IFN- $\beta$  on relative antiviral, antiproliferative and immunostimulating activities.

Starting vector: pl/24. as above

pl/24



A synthetic oligonucleotide (Chart 2q) was inserted between the DdeI\* and HgaI\* sites of pl/24 to give the nucleotide sequence shown in Chart 3i. IFNX404 is expressed from plasmid pXX404. The ~546bp BglII-BamHI fragment is then deleted to obtain high level expression.

Expression of novel, modified IFNs in Escherichia coli

All the above mentioned plasmids were grown in E.coli HB101 in the presence of a low level of tryptophan to an OD<sub>600</sub> of 0.5, then induced for IFN synthesis. The medium (200ml) contained: M9 salts, 0.5% glucose, 0.1mM CaCl<sub>2</sub>, 0.5%

Casamino acids. 1mM  $\text{MgSO}_4$ , 0.1mg/ml vitamin  $\text{B}_1$ , 2.5 $\mu\text{g}$ /ml tryptophan and 100 $\mu\text{g}$ /ml carbenecillin.

200ml of medium was inoculated with 2-4ml of an overnight culture of each clone (in the host *E.coli* HB101) grown in the above medium except for the presence of 42.5 $\mu\text{g}$ /ml tryptophan and grown at 37°C with vigorous aeration. At  $\text{OD}_{600}$  of 0.5, indole acrylic acid the inducer of the *E.coli* *trp* promoter and therefore also of IFN synthesis. was added to 20 $\mu\text{g}$ /ml. At 4-5 hours after induction 16ml of culture was withdrawn ( $\text{OD}_{600}$ =0.75-1.2 range) and split as follows: 1) 1ml was for estimation of total "solubilized" IFN antiviral or antiproliferative activity (the activity regained after a denaturation/renaturation cycle); and 2) 1ml was for display of the total accumulated *E.coli* proteins plus IFN in a polyacrylamide gel.

a) Estimation of TOTAL "solubilized" IFN antiviral activity

For recovery of TOTAL "solubilized" IFN antiviral activity. the pellets were vortexed in 20 $\mu\text{l}$  "lysis buffer" per 0.1  $\text{OD}_{600}$  per ml of culture. ("Lysis buffer" is 5M urea, 30mM NaCl, 50mM Tris-HCl pH7.5, 1% SDS, 1% 2-mercaptoethanol. 1% HSA). The mixture was heated for 2-3 min. at 90°C, frozen at -70°C for 15 min., thawed and centrifuged at 17K rpm for 20 min. The supernatant was diluted in 1 log steps to 1:10<sup>5</sup>. and appropriate dilutions immediately assayed for IFN antiviral activity by

monitoring the protection conferred on Vero cells against the cytopathic effect (cpe) of EMC virus in an in\_vitro micro-plate assay system (e.g see Dahl and Degre. Acta. Path. Microbiol. Scan . 1380, 863. 1972). The diluent was 50mM Tris-HCl pH7.5. 30mM NaCl. 1% human serum albumin (HSA).

b) Polyacrylamide gel electrophoresis of total polypeptides

Cells from 1ml of culture were mixed with 10 $\mu$ l per 0.1 OD<sub>600</sub> per ml of final sample buffer: 5M urea, 1% SDS, 1% 2-mercaptoethanol. 50mM Tris-HCl pH7.5, 30mM NaCl and 0.05% bromophenol blue. The mixture was heated at 90°C for 5 min., centrifuged for 10 min. and 5-7 $\mu$ l loaded on a 15% acrylamide/0.4% bisacrylamide "Laemmli" gel. Electrophoresis was at 70V for 18 hours. The gel was fixed and stained with Coomassie brilliant blue. then dried and photographed.

Purification and biological properties of TPNX407, X408 and X415

One litre culture was induced and grown to OD<sub>600</sub> 1-2 as described above. The cell pellet was resuspended in 30ml 50mM Tris-HCl pH8.0 and sonicated on ice. 4 x 1 min at 100W and then centrifuged for 1hr at 15K rpm. 30ml boiling extraction solution (50mM Tris-HCl pH8.0, 50mM DTT and 1-2% SDS) was added. mixed and the solution was sonicated. The solution was then boiled for 5 min., centrifuged for 1hr at 15K rpm. and to the supernatant was added (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> to 40% saturation. After 15 min. the precipitate was collected by centrifugation at 10K rpm for 20 min. The pellet was

redissolved by adding 5ml warm 50mM Tris-HCl pH8.0.

Following a 15K rpm spin for 1hr. the solution was re-reduced in 50mM DTT by boiling for 5 min.

The IFNs were fractionated on a 2.35cm x 70cm column of LKB Aca44 in 0.1% SDS, 50mM Tris-HCl pH8.0. and the peak fractions containing 1-2mg IFN were pooled.

To remove SDS and deplete pyrogens, either a) the protein was acetone precipitated and redissolved in 50% formic acid- 10% isopropyl alcohol (solvent A); or b) 6 parts formic acid and 1 part isopropyl alcohol were premixed and added to 3 parts sample. The mixture was applied to C-18 Sep-Pak TM (capacity greater than 3mg) or to a C-18 Bond Elut (Anachem). The columns were first washed with solvent A (2-4ml) and the IFN eluted with 50% formic acid 50% isopropyl alcohol.

The eluted IFN was dialysed against water to remove formate and then into GuHCl (6M)- 100mM Tris-HCl pH8.0. To renature the IFN. the sample was reduced in 10mM DTT at 100°C. then diluted 100-fold into 100mM Tris-HCl pH8.0, 200mM KCl. 1mM EDTA and either 0.1% Tween-20 or 1% BSA. Protein was estimated prior to biological assay.

#### Antiviral assays of purified, modified interferons

A single virus (encephalomyocarditis -EMC) was used to determine antiviral activity in primate cells. Determinations were made with a virus cytopathic effect (cpe) assay following challenge of cells of Monkey (Vero) and human

(Chang conjunctiva and Searle 17/1 fibroblast) origin (Dahl and Degre. *ibid.*).

Antiproliferative assays of purified, novel interferons

Antiproliferative activity was assessed by the ability of the IFN to inhibit the replication of three human cell lines (Horoszewicz et al. *Science*, 206. 1091, 1979) - Daudi (lymphoblastoid), HEP-2 (carcinoma) and RD (rhabdomyosarcoma). Daudi cells (in log phase) were cultured for 6 days in 96 well plates in the presence of various dilutions of interferon. The phenol red indicator in the medium changes from red to yellow (more acid) with progressive cell growth. Liquid paraffin was added to prevent pH change on exposure to the atmosphere and the pH change in the medium measured colorimetrically on a Dynatech plate reader. Interferon inhibition of cell growth is reflected by a corresponding reduction in the colour change. HEP-2 and RD in log growth were cultured for 3 days in 96 well plates in the presence of interferon. The cells were then fixed with 0.25% glutaraldehyde and stained with methylene blue. After extraction into ethanol the colour intensity was measured on a Dynatech plate reader. Once again colour intensity can be related proportionally to cell growth. In vitro antiproliferative activity of the novel, modified IFNs in crude bacterial extracts was also measured (Daudi cell line only).

Stimulation of Antibody-Dependent Cellular Cytotoxicity by  
novel, modified interferons (ADCC)

ADCC represents a cellular system which is immunologically specific. the effect being mediated by antibody. There are several possible versions of this assay. <sup>51</sup>Cr-labelled human red cells (GpA. Rh +ve) sensitised with anti-A antibody using the serum from a Group O individual were incubated with buffy coat cells from a Group O individual. Interferon was assessed by prior overnight incubation with buffy coat cells and its effects compared with those of parallel untreated controls (McCullagh et al. J. IFN Res., 3, 97, 1983).

Comparison of IFN protein expression. antiviral activity and  
antiproliferative activity in bacterial extracts

Table 1 sets out the expression levels and antiproliferative and antiviral activities of the group II novel, modified IFNs in crude bacterial extracts. A range of activities may be given reflecting natural variation in a biological system or assay. The activity quoted is that which is regained after SDS/urea/mercaptoethanol treatment. by diluting the extract in 1% human serum albumin. as above.

It may be seen in Table 1 that for the control, IFN- $\beta$ , antiviral (AV) and antiproliferative (AP) activity vary over not more than a 4-fold range (>20 experiments). Examples of reduced AV activity in relation to AP activity and expression are IFNX407 and IFNX419 when compared with IFN- $\beta$ . IFNX407 has ~2 to 9-fold lower AV activity and a <2-fold lower AP activity than IFN- $\beta$ . An even greater differential is

displayed by IFNX419. which has 7.7 to 57-fold lower AV activity than IFN- $\beta$ , and a virtually unchanged AP activity.

Table 1

Novel, modified interferon	IFNX No.	Expression (% of total cell protein)	EMC/Vero cell - Antiviral activity IU/Litre/OD <sub>680</sub>	Daudi cell - Anti-proliferative activity U/ml=dilution at IC <sub>50</sub> *
$\beta^{9-56} \rightarrow \alpha_1^{7-54}$	407	5-10	$2.3 \times 10^7$	$1.9 \times 10^3$
$\beta^{44-56} \rightarrow \alpha_1^{42-54}$ [Ser <sup>17</sup> ]	408	3	$0.46-2.0 \times 10^7$	$1.1-1.6 \times 10^3$
$\beta^{42-56} \rightarrow \alpha_1^{40-54}$ [Ser <sup>17</sup> ]	409	3	$0.4-1.0 \times 10^7$	$<10^3$
$\beta^{2-7} \rightarrow \alpha_2^{1-5}$	410	10-20	$3.3-7.2 \times 10^7$	$2 \times 10^3$
$\beta^{9-56} \rightarrow \alpha_1^{7-54}$				
$\beta^{28-46} \rightarrow \alpha_1^{28-46}$	415	10-15	$1.9 \times 10^9$	$1.5 \times 10^5$
[Cys <sup>17</sup> $\rightarrow$ Ser] [Met <sup>31</sup> $\rightarrow$ Lys]				
$\beta^{9-42} \rightarrow \alpha_1^{7-40}$	419	7	$3.5-6.5 \times 10^6$	$2.6 \times 10^3$
$\beta^{21-42} \rightarrow \alpha_1^{19-40}$	420	$<1.5$	$6.8-7.7 \times 10^6$	$<10^3$
[Cys <sup>17</sup> $\rightarrow$ Ser]				
IFN- $\beta$ control	-	10	$0.5-2.0 \times 10^8$	$3.4 \times 10^3$

n.d. = not done

\* U/ml = Dilution of sample assayed for antiviral activity giving 50% inhibition of cell growth



In conclusion, the novel, modified IPNs present in bacterial extracts display marked differences in relative AP and AV activity when compared with each other and with IPN- $\beta$ . To determine more precisely the differences in biological activity between the novel IPNs and IPN- $\beta$ , certain of the above examples were subjected to protein purification. The following assays were also designed to determine whether any of the novel IPNs displayed altered cell specificity.

The in vitro antiviral. antiproliferative and  
immunostimulating (ADCC) activities of purified novel,  
modified IFNs

a) Antiviral

Table 2 compares the in vitro antiviral activity of purified IFNX415 with IFN- $\beta$  and IFNX805, against EMC virus in three different cell lines. IFNX415 displays an increase in antiviral activity only in the CHANG cell line (6 to 7-fold). Thus the antiviral specificity of this novel, modified IFN may be different from that of IFN- $\beta$  and IFNX805. Table 3 gives the result of a separate experiment in which purified IFNX407 and IFNX408 are compared with IFN- $\beta$ . IFNX407 has substantially lower specific antiviral activity than IFN- $\beta$  on all three cell lines, while IFNX408 is similar to IFN- $\beta$ .

Table 2**Antiviral Activity of Purified Novel, Modified Interferons  
(Units/mg IFN Protein)**

IFNI No.	CELL LINE		
	17/1	Chang	Vero
X415	$1.8 \times 10^5$	$3.1 \times 10^6$	$3.3 \times 10^5$
BETA	$1.3 \times 10^5$	$5.1 \times 10^5$	$7.6 \times 10^5$
X805	$7.6 \times 10^4$	$4.4 \times 10^5$	$4.2 \times 10^5$
RATIOS			
X415/BETA	1.4	6.1	0.4
X415/X805	2.4	7.0	0.8
X805/BETA	0.6	0.9	0.6

Table 3

Antiviral activity of purified novel. modified interferons

Units/mg IFN protein

IFNX No.	CELL LINE		
	17/1	CHANG	VERO
X407	$<5.1 \times 10^3$	$9.5 \times 10^3$	$1.9 \times 10^4$
X408	$4.2 \times 10^5$	$4.6 \times 10^5$	$1.2 \times 10^6$
BETA	$1.9 \times 10^5$	$7.2 \times 10^5$	$9.1 \times 10^5$
RATIOS			
X407/BETA	$<0.02$	0.01	0.02
X408/BETA	2.2	0.6	1.3

b) Antiproliferative

Table 4 compares the in vitro antiproliferative activity of purified IFNX415 with IFN- $\beta$  and IFNX805, on three different transformed cell lines IFNX805 and IFN- $\beta$  display similar activities in all three cell lines, while IFNX415 has a significantly increased activity in the Daudi cell. Thus the in vitro antiproliferative specificity of IFNX415 may be different from that of IFN- $\beta$  and IFNX805. Table 5 gives the result of a separate experiment in which purified IFNX407 and IFNX408 are compared with IFN- $\beta$ . IFNX407 has substantially lower antiproliferative activity than IFN- $\beta$  against HEP-2 and RD cell lines but similar activity to IFN- $\beta$

148

35  
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against the Daudi cell line IFNX408 is similar  
to IFN- $\beta$ .

Table 4

**Antiproliferative Activity of Purified Novel. Modified  
Interferons**

(Units/mg IFN Protein)

IFNX No.	CELL LINE		
	REP-2	RD	DAUDI
X415	$2.2 \times 10^4$	$2.2 \times 10^4$	$1.9 \times 10^5$
BETA	$8.9 \times 10^3$	$6.5 \times 10^3$	$1.4 \times 10^4$
X805	$5.4 \times 10^3$	$4.3 \times 10^3$	$1.2 \times 10^4$
RATIOS			
X415/BETA	2.5	3.4	13.6
X415/X805	4.1	5.1	15.8
X805/BETA	0.6	0.7	0.9

Table 5

**Antiproliferative activity of purified novel, modified  
interferons**

Units/mg IFN protein

IFNX No. ....	CELL LINE		
	HEP-2	RD	DAUDI
X407	$<3.6 \times 10^1$	$<2.0 \times 10^2$	$1.0 \times 10^5$
X408	$8.9 \times 10^3$	$1.0 \times 10^4$	$3.1 \times 10^5$
BETA	$1.3 \times 10^4$	$1.9 \times 10^4$	$2.5 \times 10^5$
RATIOS			
X407/BETA	$<0.002$	$<0.01$	0.4
X408/BETA	0.7	0.5	1.2

c) Immunostimulating (ADCC)

Table 6 compares the in vitro activity of purified IFNX415 with IFN- $\beta$  and IFNX805, as an effector of Antibody-Dependent Cellular Cytotoxicity (ADCC) against human red cells. Overall, IFNX415, IFNX805 and IFN- $\beta$  did not differ significantly in their ability to stimulate ADCC activity of buffy coat preparations from 5 group 0 donors. This is in contrast with the increased AV activity in CHANG cells and the increased AP activity in Daudi cells of IFNX415 (Tables 2 and 3). Table 7 gives the result of a separate experiment in which IFNX407 and IFNX408 are compared. The ADCC activity of IFNX407 was too low for accurate assessment

150

IFNX488 had slightly reduced specific activity  
compared with IFN- $\beta$ .

Table 6

**Immunomodulatory (ADCC) Activity of Purified Novel, Modified  
Interferons**

(Units/mg IFN Protein)					
IFNX No.	DONOR				
	1	2	3	4	5
X415	$1.3 \times 10^3$	$2.3 \times 10^3$	$7.1 \times 10^2$	$6.3 \times 10^3$	$1.2 \times 10^3$
BETA	$1.0 \times 10^3$	$1.9 \times 10^3$	$1.1 \times 10^2$	$1.1 \times 10^4$	$1.7 \times 10^3$
X885	$4.5 \times 10^2$	$3.2 \times 10^2$	$1.5 \times 10^2$	$1.8 \times 10^3$	$5.8 \times 10^2$
RATIOS					
X415/BETA	1.3	1.2	6.5	0.6	0.7
X415/X885	2.9	7.2	4.7	3.5	2.1
X885/BETA	0.5	0.2	1.4	0.2	0.3

Table\_7

Immunomodulatory (ADCC) activity of purified novel, modified interferons

<u>Units/mg_IPN Protein</u>						
DONOR						
IPNX No.	1	2	3	4	5	6
X407	$<10^2$	$<10^2$	$<10^2$	$<10^2$	$<10^2$	$<10^2$
X408	$1.8 \times 10^2$	$6.2 \times 10^3$	$2.6 \times 10^2$	$1.3 \times 10^2$	$1.6 \times 10^3$	$9.1 \times 10^2$
BETA	$1.5 \times 10^3$	$2.4 \times 10^4$	$9.3 \times 10^2$	$3.0 \times 10^3$	$4.0 \times 10^3$	$2.6 \times 10^3$
RATIOS						
X407/BETA	$<0.06$	$<0.004$	$<0.1$	$<0.03$	$<0.02$	$<0.04$
X408/BETA	0.1	0.3	0.3	0.4	0.4	0.4

Purification and Biological Properties of IPNX402 and IPNX404

1. Methods

The expressed IFN proteins were extracted from E.coli with the aid of sodium dodecyl sulphate (SDS) and purified by chromatography on AcA44. IPNX401 had estimated purities of 70-90% based on polyacrylamide gel electrophoretic (PAGE) analysis.

The novel interferons were tested to determine its specific antiviral, antiproliferative and immunomodulatory activities. The following assay systems were employed:



1) Antiviral assay

- (a) Cytopathic (CPE) assay with encephalomyocarditis (EMC) virus. This is a standard assay which measures the ability of interferon to protect cell monolayers against the cytopathic effect of EMC virus. The cell lines used were: Vero (African Green Monkey epithelial), WISH (amnion epithelial), MRC-5 (foetal lung fibroblast) and 17-1 (foetal lung fibroblast). Cell monolayers were established in 96 well flat-bottomed microtitre plates in DMEM medium containing 2% donor calf serum plus glutamine and antibiotics. Serial 1 in 2 dilutions of interferon were incubated with the cells at 37° for 18-24 hours. the supernatant discarded and an appropriate challenge dose of EMC virus in medium added. After incubation at 37° for a further 24 hours, the supernatants were discarded the monolayers fixed with formol/saline and stained with crystal violet. The plates were read visually to establish the dilution of interferon giving 50% inhibition of the cytopathic effect.
- (b) Plaque reduction assay - using Herpes simplex type 2 (HSV-2) virus with Vero (monkey) Chang (human) and MDBK (bovine cells). Confluent monolayers of cells were established in 96 well flat-bottomed microtitre plates. After incubation at 37° for 18 hours with dilutions of interferons. the cells were challenged with an appropriate number of plaque forming units of

virus, overlaid with medium containing 0.5% carboxymethyl cellulose and incubated at 37° for 24 hours. After fixation and staining the plaques were counted microscopically and the counts expressed as a percentage of the mean maximum plaque counts in untreated control wells. Interferon titres are the reciprocal dilutions giving 50% reduction in plaque number/well.

ii) Antiproliferative assay

Daudi cells in Dulbecco's Modified Eagles Medium (DMEM) were seeded at  $2 \times 10^5$ /ml (200  $\mu$ l) in 96 well tissue culture plates. Interferons were added at the time of seeding and cells incubated at 37° in a humidified 5% CO<sub>2</sub> atmosphere. After 22 hours tritiated thymidine was added and the cells incubated for a further 2 hours after which they were harvested on a Flow cell harvester washed and treated with 5% trichloroacetic acid. Acid insoluble radioactivity was counted and inhibition of thymidine incorporation was taken as a measure of the antiproliferative activity of interferon.

iii) Immunomodulatory assay (Natural Killer (NK) Cell Activity)

Buffy coat cells separated from human peripheral blood by Ficoll/Hypaque sedimentation were suspended in supplemented RPMI 1640 medium and incubated overnight at 37° with interferon

dilutions After washing to remove interferon. these effector cells were incubated at 37° for a further 4 hours with <sup>51</sup>Cr-labelled K562 cells at effector to target cell ratios of 20:1 or 10:1. (K562 is a human tumour-derived cell line). After centrifugation an aliquot of the supernatant was removed for measurement of released radioactivity. Maximum <sup>51</sup>Cr release was obtained by repeated freeze-thawing of a target cell suspension and a background control obtained by measurement of <sup>51</sup>Cr release from target cells incubated without effector cells. Results were expressed as percentage specific <sup>51</sup>Cr release:

$$\frac{\text{Test sample} - \text{background}}{\text{Maximum release} - \text{background}} \times 100$$

## 2. Results

### 1) Antiviral activities

#### (a) CPE assay - EMC virus

Table 8 lists the assay means for hybrid X401 and the recombinant-derived IFN-β measured against EMC virus in Vero and the four human cell lines. The activities are expressed in units/mg protein

From the individual interferon means in different cell types contained in Table 8 and from the summary pooled data across all cell

types it is seen that IFNX402 and IFNX404 have consistently lower activities than IFN- $\beta$ , the reduction being most severe with IFNX402. The pooled mean antiviral activity shown in the analysis of variance for IFNX402 is less than 1% that of IFN- $\beta$ . The activity of IFNX404 is 5-15% of IFN- $\beta$ .

Table 8

Antiviral activities of recombinant interferons against encephalomyocarditis virus (IFN units/mg protein)

Mean activities in each cell line

PREPARATION	CELL LINE					
		Vero	Chang	WISH	MRC-5	17-1
IFN- $\beta$	x	$1.5 \times 10^5$	$5.2 \times 10^5$	$8.4 \times 10^5$	$1.5 \times 10^5$	$7.1 \times 10^4$
IFNX402	x	$2.5 \times 10^3$	$4.1 \times 10^2$	$4.8 \times 10^3$	$4.9 \times 10^2$	$3.3 \times 10^2$
IFNX404	x	$2.6 \times 10^4$	$1.8 \times 10^4$	$4.8 \times 10^4$	$5.8 \times 10^3$	$2.3 \times 10^3$

( $\bar{x}$  calculated based upon 3-5 assays)

PREPARATION	POOLED MEAN	95% CONFIDENCE LIMITS (u/mg)
IFN- $\beta$	$2.4 \times 10^5$ u/mg	$1.5-3.9 \times 10^5$
IFNX402	$1.1 \times 10^3$ u/mg	$0.6-2.1 \times 10^3$
IFNX404	$8.9 \times 10^3$ u/mg	$5.4-14.8 \times 10^3$

For comparative purposes, the observed activities (in units/ml) of preparations of fibroblast IFN- $\beta$  and leucocyte IFN- $\alpha$  are shown

in Table 9. These natural interferons were not available in purified form and were used in the assays in dilute solutions containing large amounts of non-interferon protein. Thus, results with natural IFN- $\beta$  and IFN- $\kappa$  cannot be quoted in units/mg and the results in Table 9 are not directly comparable with those of Table 8. Nevertheless, it can be seen that the activity of both natural interferons is sustained across the five cell lines within an interferon class with the exception that WISH cells appear slightly more sensitive to both IFN- $\beta$  and IFN- $\kappa$ .

Table 9

Relative antiviral activities of natural interferon preparations against encephalomyocarditis virus in vero and human cell lines

PREPARATION	<u>Interferon units/ml</u>				
	CELL LINE				
	Vero	Chang	WISH	MRC-5	17-1
Fibroblast-derived $\beta$ x	$3.6 \times 10^4$	$5.6 \times 10^4$	$1.3 \times 10^5$	$7.8 \times 10^4$	$6.8 \times 10^4$
Leucocyte-derived IFN- $\kappa$ x	$2.5 \times 10^2$	$1.5 \times 10^2$	$1.3 \times 10^3$	80	80

(b) Plaque reduction assays HSV-2

Similar estimates of antiviral activities obtained with HSV-2 by means of plaque reduction assays are given in Table 10. In this case the experiments were confined to the human Chang cells, primate Vero cells on bovine MDBK cells. IFNX402 and IFNX404 show reduced antiherpes activity in human and monkey cells. With the bovine cell line, IFNX404 shows reduced activity as it does in Chang and Vero, but surprisingly, the activity of IFNX402 in this cell line is unchanged from that of the IFN- $\beta$  parent. An analysis of variance confirmed that the observed reduction in activity for IFNX402 and X404 in Vero and Chang cells is highly significant as is the difference between the activity of IFNX402 in Chang and MDBK cells.

The pattern of natural IFN- $\beta$  and IFN- $\kappa$  against HSV-2 in these 3 cell lines is shown in Table 11. again expressed as units/ml rather than as specific activity as a result of impure IFNs. In contrast to some reported results from other laboratories, IFN- $\beta$  reacts reasonably well with our MDBK cell line, producing antiviral activity at about the same dilution as Vero or Chang cells. On the other hand, the IFN- $\kappa$  standard reacted substantially better with MDBK cells than with either Vero or Chang cells. In view of this control data, the reduced activity of IFNX402 in Chang and Vero cells and retained activity in MDBK

cells represents a pattern of response in these cell lines which is similar to that obtained with natural IFN- $\alpha$ .

Table 10

Antiviral activities of recombinant interferons against HSV-2 determined by plaque reduction assay

Interferon units/mg protein

PREPARATION		CELL LINE		
		Vero	Chang	MDBK
IFN- $\beta$	x	$1.2 \times 10^5$	$4.7 \times 10^5$	$2.5 \times 10^5$
IFNX402	x	$1.1 \times 10^3$	$1.3 \times 10^3$	$3.4 \times 10^5$
IFNX404	x	$7.0 \times 10^3$	$9.9 \times 10^3$	$4.4 \times 10^3$

Table 11

Relative antiviral activity of natural interferons against RSV-2 in monkey, human and bovine cells determined by plaque reduction

Interferon units/ml

PREPARATION		CELL LINE		
		Vero	Chang	MDBK
Fibroblast-derived IFN- $\beta$	x	$2.6 \times 10^4$	$9.3 \times 10^4$	$1.9 \times 10^4$
Leucocyte-derived IFN- $\alpha$	x	59	90	$6.8 \times 10^3$

Summarizing the results of antiviral activity with RNA and DNA viruses in relevant cell types. Table 12 lists the activities of the recombinant and natural interferons against EMC and HSV-2 in Chang and Vero cells (data from Tables 8-11). There is no indication from these results of preferential activity of IFNX402 against one or other of the 2 virus types. The results from the 2 sets of assays are remarkably similar and are not significantly different. Thus the pooled mean antiviral activity against EMC virus shown in the analysis of variance to Table 8 is equally valid as an estimate of antiherpes activity and can be used as an overall indicator of specific antiviral activity of IFNX402.

Table 12

Relative antiviral activity against encephalomyocarditis virus and HSV-2 for recombinant and natural interferons assayed in human and monkey cells

Recombinant interferons (unit/mg protein)

IFN Preparation	Pooled mean activity EMC virus (from Table 1 analysis)	Pooled mean activity HSV-2 Vero and Chang cells
IFN- $\beta$	$2.4 \times 10^5$	$3.5 \times 10^5$
IFNX402	$1.1 \times 10^3$	$1.2 \times 10^3$
IFNX404	$8.9 \times 10^3$	$8.5 \times 10^3$

160



(c) Comparative antiviral\_data\_with an atypical  
Chang cell\_line

One line of Chang conjunctival cells maintained in high passage (approx. X 160) has undergone a mutational change such that it is approximately 3 times more sensitive to IFN- $\beta$  than the normal low passage Chang cells which we have used in routine assays. At the same time the atypical high passage Chang cells recognize and respond to IFN- $\alpha$  with a 100-fold increase in sensitivity compared to the parental low passage Chang cells.

Comparative ratios of antiviral activity in high and low passage Chang cells can therefore be used to indicate a degree of  $\alpha$ -like property in a particular recombinant.

The results of profiling the recombinant IFNX molecules in this way is shown in Table 13. IFNX402 is prominent in demonstrating  $\alpha$ -like activity.

ii) Antiproliferative\_activity

The various recombinant interferons were assayed for growth inhibitory activity against Daudi lymphoblastoid cells. in at least 4 replicate assays with each interferon. The mean results of these assays are given in Table 14, activities being expressed as the protein concentration

required to produce a 50% inhibition of maximum thymidine incorporation in untreated control cells (Inhibitory Dose<sub>50</sub>). The poorly antiviral IFNX402 can be seen to have an identical growth regulating activity to the IFN- $\beta$  parent. In contrast IFNX404 has lost growth regulating activity.

Table 13

Antiviral activities of recombinant and natural  
interferons in atypical Chang cells

	Chang <sup>A</sup> (High passage)	Chang (Routine low passage)	Ratio ChA/Ch
<u>Units/mg</u>			
IFN- $\beta$	$1.6 \times 10^6$	$5.2 \times 10^5$	3
IFNX402	$1.3 \times 10^6$	$4.1 \times 10^2$	3170
IFNX404	$5.3 \times 10^4$	$1.8 \times 10^4$	3
<u>Units/ml</u>			
Fibroblast IFN- $\beta$	$1.7 \times 10^5$	$5.6 \times 10^4$	3
Leucocyte IFN- $\alpha$	$3.4 \times 10^4$	$1.5 \times 10^2$	226

Table 14Antiproliferative activity of recombinant interferons  
assayed in Daudi human lymphoblastoid cellsInhibitory Dose<sub>50</sub> (µg/ml)

PREPARATION	No. of replicate assays (n)	Corrected Mean ID <sub>50</sub>	95% Confidence Limits
IFN-β	4	3.8	1.5-9.8
IFNX402	6	3.2	1.4-6.9
IFNX404	4	44.7	17.4-114.8

iii) Immunomodulatory activity - NK assay

The recombinant interferons were also repeatedly assayed for ability to enhance natural killer (NK) cell activity, a total of 9-11 assays contributing to the results which are shown in Table 15. In a similar fashion to the antiproliferative activity, the specific NK stimulating activity is expressed as the protein dose concentration producing a 50% effect (Stimulating Dose<sub>50</sub>).

IFNX402 has substantially lost NK stimulating activity, being about 35-fold less active than IFN-β parent. IFNX404 has also less activity but only by a factor of 4. These differences are significant as shown in the analysis of variance.

Immunostimulant activities of recombinant interferons  
assayed with human NK cells

PREPARATION	No. of replicate assays (n)	Corrected Mean SD <sub>50</sub>	95% Confidence Limits
IFN- $\beta$	11	3.4	2.1-5.4
IFNX402	9	117.0	339
IFNX404	10	15.1	9.3-24.5

3. Conclusions

Mean specific activities for the antiviral, antiproliferative and immunomodulatory properties of each interferon are summarized in Table 16. (It should be noted that activity varies directly with the figures taken from antiviral assays but inversely with the figures quoted from ID<sub>50</sub> and SD<sub>50</sub> assays). For convenience these results have been indexed relative to the IFN- $\beta$  parent in the lower half of Table 16.

Table 16Comparative summary of biological data for recombinant and natural interferons

PREPARATION	Specific antiviral activity ( $\mu$ /mg)	Specific antiproliferative activity ( $ID_{50}$ $\mu$ g/ml $^{-1}$ )	Specific immunostimulant activity ( $SD_{50}$ $\mu$ g/ml $^{-1}$ )
IFN- $\beta$	$2.4 \times 10^5$	3.8	3.4
IFNX402	$1.1 \times 10^3$	3.2	195.0
IFNX404	$8.9 \times 10^3$	44.7	15.1
Indexed results (IFN- $\beta$ = 100)			
IFN- $\beta$	100	100	100
IFNX402	0.5	100 (118)	3
IFNX404	3.7	9	23

Figures in brackets indicate actual calculated index where it is not significantly different from 100. In all other cases, differences from 100 are significant.

IFNX402 has identical antiproliferative activity to IFN- $\beta$  but has dramatically reduced antiviral and immunostimulant activities. IFNX404 has reduced efficacy in all 3 classes of biological assay.

The most surprising result to come out of these analyses is the separation of activities achieved with IFNX402. This finding was unexpected but it is of great interest since the presence of full antiproliferative activity in a molecule which has major reductions in its other properties might be

effective as an anti-tumour agent but have reduced toxicity and lack unwanted side effects.

The major conclusions summarized above are based on results in human cell systems. MDBK (bovine) and Chang<sup>A</sup> (atypical human) showed increased sensitivity to IFNX402 such that the decrease in antiviral properties of this hybrid seen in normal human cells are not seen with these heterologous or atypical systems. As a result, the ratio in antiviral activity between MDBK and human or monkey cells (Table 10) or between Chang<sup>A</sup> and the normal Chang cells (Table 13) is dramatically large with the IFNX402 preparation and clearly different from that of IFN- $\beta$  or any other hybrids. An elevation in the MDBK/Chang or Chang<sup>A</sup>/Chang ratio is characteristic of natural leucocyte IFN- $\alpha$  (Tables 11 and 13). In this respect the IFNX402 hybrid has an "alpha-like" profile.

#### Pharmaceutical formulation and administration

The novel, modified interferons of the present invention can be formulated by methods well known for pharmaceutical compositions, wherein the active interferon is combined in admixture with a pharmaceutically acceptable carrier substance, the nature of which depends on the particular mode of administration being used. Remington's Pharmaceutical Sciences by E W. Martin, hereby incorporated by reference, describes compositions and formulations suitable for delivery

of the interferons of the present invention. For instance, parenteral formulations are usually injectable fluids that use physiologically acceptable fluids such as saline, balanced salt solutions, or the like as a vehicle. Oral formulations may be solid, e.g. tablet or capsule, or liquid solutions or suspensions.

The novel, modified interferons of the invention may be administered to humans or other animals on whose cells they are effective in various ways such as orally, intravenously, intramuscularly, intraperitoneally, intranasally, intradermally or subcutaneously. Administration of the interferon composition is indicated for patients with malignancies or neoplasms, whether or not immunosuppressed or in patients requiring immunomodulation, or antiviral treatment. Dosage and dose rates may parallel those employed in conventional therapy with naturally occurring interferons - approximately  $10^5$  to  $10^8$  units daily. Dosages significantly above or below these levels may be indicated in long term administration or during acute short term treatment. A novel, modified interferon may be combined with other treatments or used in association with other chemotherapeutic or chemopreventive agents for providing therapy against the above mentioned diseases and conditions, or other conditions against which it is effective.

Modifications of the above described modes for carrying out the invention such as without limitation, use of alternative vectors, alternative expression control systems, and alternative host micro-organisms and other therapeutic or

related uses of the novel interferons. that are obvious to those of ordinary skill in the biotechnology, pharmaceutical. medical and/or related fields are intended to be within the scope of the following claims.



- 1 -  
- 51 -**Claims:**

1. A modified beta interferon comprising the amino acid sequence of beta interferon wherein three to fifty-six of the beta interferon amino acids 1 to 56 are replaced by three to fifty-six other amino acids.
2. A modified beta interferon of claim 1 wherein the beta interferon amino acids replaced are 2 to 7 and 9 to 56
3. A modified beta interferon of claim 1 wherein the beta interferon amino acids replaced are 9 to 56.
4. A modified beta interferon of claim 3 wherein four to forty-seven sequential amino acids of the beta interferon amino acids 9 to 56 are replaced by four to forty-seven sequential amino acids selected from an alpha interferon's amino acids 7 to 54.
5. A modified beta interferon of claim 3 wherein four to fifteen sequential amino acids of the beta interferon amino acids 42 to 56 are replaced by four to fifteen sequential amino acids selected from an alpha interferon's amino acids 40 to 54.

6. A modified beta interferon of claim 3 wherein four to thirty-three sequential amino acids of the beta interferon amino acids 9 to 42 are replaced by four to thirty-three sequential amino acids selected from an alpha interferon's amino acids 7 to 40.
7. A modified beta interferon of claim 3 wherein four to nineteen sequential amino acids of one beta interferon amino acids 28 to 46 are replaced by four to nineteen sequential amino acids selected from an alpha interferon's amino acids 28 to 46.
8. A modified beta interferon of claim 2 wherein the beta interferon amino acids 2 to 7 and 9 to 56 are replaced sequentially by the alpha 2 interferon amino acids 1 to 5 and 7 to 54.
9. A modified beta interferon of claim 4 wherein the beta interferon amino acids 9 to 56 are replaced by alpha interferon's amino acids 7 to 54.
10. A modified beta interferon of claim 5 wherein the beta interferon amino acids 42 to 56 are replaced by alpha 1 interferon's amino acids 40 to 54.
11. A modified beta interferon of claim 6 wherein the beta interferon amino acids 9 to 42 are replaced by alpha 1 interferon's amino acids 7 to 40.

12. A modified beta interferon of claim 6 wherein the beta interferon amino acids 21 to 40 are replaced by alpha 1 interferon's amino acids 19 to 40.
13. A modified beta interferon of claim 7 wherein the beta interferon amino acids 28 to 46 are replaced by alpha 1 interferon's amino acids 28 to 46.
14. A modified beta interferon of claim 1 wherein the beta interferon amino acids 10 to 56 are replaced by alpha 1 interferon's amino acids 1 to 53.
15. A modified beta interferon of claim 3 wherein the alpha interferon are of human origin
16. A modified beta interferon of claim 1 wherein the beta interferon are of human origin.
17. A modified beta interferon of claim 4 wherein both the alpha and beta interferons are of human origin.
18. A modified beta interferon of claim 1 wherein cysteine 17 is replaced by serine 17.
19. A modified beta interferon of claim 3 wherein cysteine 17 is replaced by serine 17
20. A modified beta interferon of claim 3 wherein leucine 15 is replaced by cysteine 15.

21. A modified beta interferon of claim 3 wherein the methionine 31 is replaced by lysine 31.
22. A modified beta interferon of claim 14 comprising the amino acid of sequence of IFNX406.
23. A modified beta interferon of claim 8 comprising the amino acid of sequence of IFNX410.
24. A modified beta interferon of claim 9 comprising the amino acid of sequence of IFNX407.
25. A modified interferon of claim 9 wherein leucine 15 is replaced by cysteine 15 comprising the amino acid sequence of IFNX402.
26. A modified interferon of claim 9 wherein the alpha 1 interferon amino acid sequence is replaced by an alpha 2 interferon amino acid sequence with leucine 15 replaced by cysteine 15 comprising the amino acid sequence of IFNX403.
27. A modified beta interferon of claim 10 comprising the amino acid sequence of IFNX404.
28. A modified beta interferon of claim 10 wherein the cysteine 17 is replaced by serine 17 comprising the amino acid sequence of IFNX408.

29. A modified beta interferon of claim 11 comprising the amino acid sequence of IFNX419.
30. A modified beta interferon of claim 12 wherein cysteine 17 is replaced by serine 17 comprising the amino acid sequence of IFNX420.
31. A modified beta interferon of claim 13 wherein cysteine 17 is replaced by serine 17 and methionine 31 is replaced by lysine 31 comprising the amino acid sequence of IFNX415.
32. A modified beta interferon according to claim 1 wherein one or more of the antiviral, cell growth regulatory, or immunomodulatory activities is substantially changed from that of unmodified beta interferon.
33. A nucleic acid sequence that codes for the synthesis of the polypeptide of claim 1.
34. A nucleic acid sequence of claim 33 wherein the nucleic acid sequence is DNA.
35. The DNA sequence of claim 34. wherein the DNA codes for the synthesis of the polypeptide IFNX402.
36. The DNA sequence of claim 34. wherein the DNA codes for the synthesis of the polypeptide IFNX403.

37. The DNA sequence of claim 34. wherein the DNA codes for the synthesis of the polypeptide IFNX404.
38. The DNA sequence of claim 34, wherein the DNA codes for the synthesis of the polypeptide IFNX406.
39. The DNA sequence of claim 34, wherein the DNA codes for the synthesis of the polypeptide IFNX407.
40. The DNA sequence of claim 34. wherein the DNA codes for the synthesis of the polypeptide IFNX408.
41. The DNA sequence of claim 34. wherein the DNA codes for the synthesis of the polypeptide IFNX409.
42. The DNA sequence of claim 34. wherein the DNA codes for the synthesis of the polypeptide IFNX410.
43. The DNA sequence of claim 34, wherein the DNA codes for the synthesis of the polypeptide IFNX415.
44. The DNA sequence of claim 32, wherein the DNA codes for the synthesis of the polypeptide IFNX419.
45. The DNA sequence of claim 32, wherein the DNA codes for the synthesis of the polypeptide IFNX420.
46. The recombinant plasmid comprising a replicating cloning vehicle in combination with the DNA sequence of claim 33.

47. A recombinant plasmid of claim 46. wherein the DNA sequence codes for the synthesis of IFNX402, 403, 404, 406, 407, 408, 409, 410, 415, 419, or 420.
48. A cell transformed by the recombinant plasmid of claim 47.
49. A process for producing a modified beta interferon comprising the growth of a cell of claim 48 and the isolation of the resulting polypeptides,
50. A pharmaceutical composition comprising an effective amount of the modified beta interferon of claim 1 admixed with a pharmaceutically acceptable carrier.
51. A pharmaceutical composition comprising an effective amount of the modified beta interferon of claim 3 admixed with a pharmaceutically acceptable carrier.
52. A pharmaceutical composition comprising an effective amount of IFNX402 admixed with a pharmaceutically acceptable carrier.
53. A pharmaceutical composition comprising an effective amount of IFNX403 admixed with a pharmaceutically acceptable carrier.

54. A pharmaceutical composition comprising an effective amount of IFNX404 admixed with a pharmaceutically acceptable carrier.
55. A pharmaceutical composition comprising an effective amount of IFNX406 admixed with a pharmaceutically acceptable carrier.
56. A pharmaceutical composition comprising an effective amount of IFNX407 admixed with a pharmaceutically acceptable carrier.
57. A pharmaceutical composition comprising an effective amount of IFNX408 admixed with a pharmaceutically acceptable carrier.
58. A pharmaceutical composition comprising an effective amount of IFNX409 admixed with a pharmaceutically acceptable carrier.
59. A pharmaceutical composition comprising an effective amount of IFNX410 admixed with a pharmaceutically acceptable carrier.
60. A pharmaceutical composition comprising an effective amount of IFNX415 admixed with a pharmaceutically acceptable carrier.



61. A pharmaceutical composition comprising an effective amount of IFNX419 admixed with a pharmaceutically acceptable carrier.
62. A pharmaceutical composition comprising an effective amount of IFNX420 admixed with a pharmaceutically acceptable carrier.
63. A method of treating viral infections comprising the administration of an effective amount of the modified beta interferon of claim 1.
64. A method of regulating cell growth comprising the administration of an effective amount of the modified beta interferon of claim 1.
65. A method of regulating the immune system comprising the administration of an effective amount of the modified beta interferon of claim 1.

FIG. 1

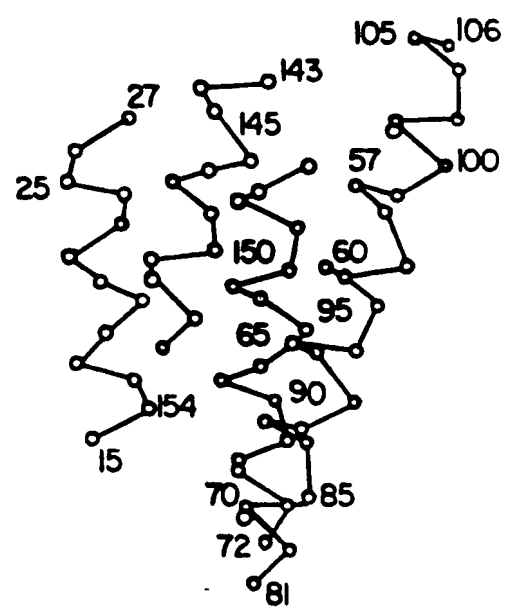
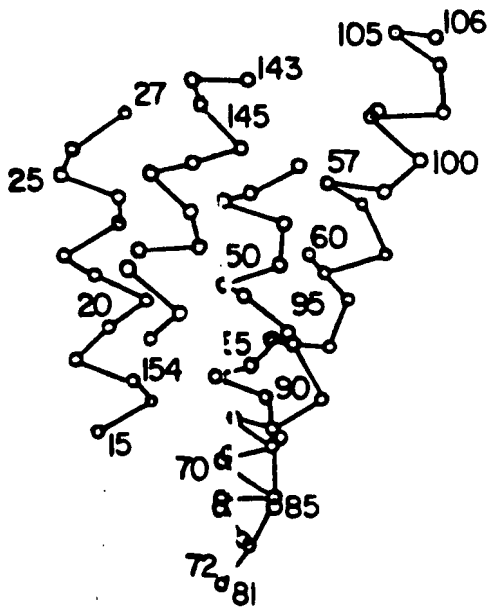
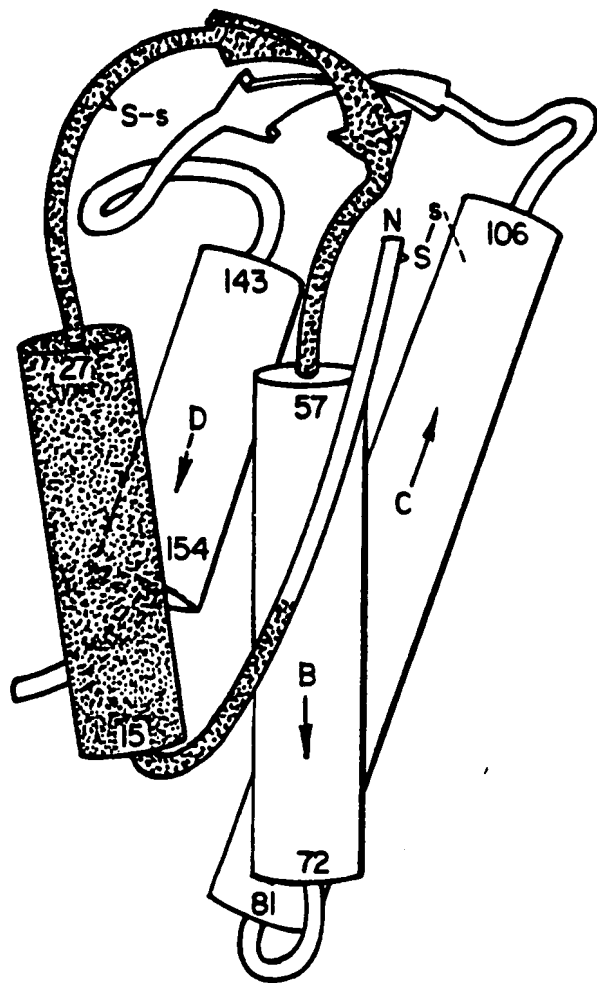


Fig. 2  
Chart 2a

0131816

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Chemically synthesized sequence for IPHX487

HinfI

ATTCCATTCTCTCGACAACAGACGTACCCTTATGCTGCTCGCTCAGATGAGCCGGATATCCCC  
GGTAAGAGAGCTGTTGTCTGCATGGGAATACGACGAGCGAGTCTACTCGGCCTATAGGGG

GTCTTCTTGCCTGATGGACCGCCACGACTTCGGCTTCCCTCAGGAAGAATTCGATGGCAATCA  
CAGAAGAACGGACTACCTGGCGGTGCTGAAGCCGAAGGGAGTCCTTCTTAAGCTACCGTTAGT

GTTTCAGAAAGCACCTGCGATTC  
CAAAGTCTTTCGTGGACGCTAAGACTGG

HgaI

Fig. 3



0131816

Chart 2b

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**Chemically synthesized sequence for IPHX418**

Clal

CGATAAGCTATGTGCGACTTACCACAATTCCATTCTCTCGACAACCGTCGTACTCTGATGCTG  
TATTCGATACACGCTGAATGGTGTAAAGGTAAGAGAGCTGTTGGCAGCATGAGACTACGAC

CTCGCTCAGATGAGCCGGATATCCCCGTCTTCTTGCCCTGATGGACCGCCACGACTTCGGCTTC  
GAGCGAGTCTACTCGGCCTATAGGGGCAGAAGAACGGACTACCTGGCGGTGCTGAAGCCGAAG

CCTCAGGAAGAATTCGATGGCAATCAGTTTCAGAAAGCACCTGCGATTCTGACCATCTACGAA  
GGAGTCCTTCTTAAGCTACCGTTAGTCAAAGTCTTTCGTGGACGCTAAGACTGGTAGATGCTT

ATGCTGCAAAACATCTTCG  
TACGACCTTTGTAGAAGC

NluI

Fig. 4  
Chart 2c

0131816

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**Chemically synthesized oligonucleotide for IFN $\alpha$ 415**

Clal

CGATAAGCTATGAGCTACAACCTGCTTGGATTCTACAAAGAAGCAGCAATTTTCAGTCTCAG  
TATTCGATACTCGATGTTGAACGAACCTAAGGATGTTTCTTCGTCGTTAAAAGTCAGAGTC

AAGCTCCTGTGGCAATTGAATGGGAGGTCTTGCCTGAAGGACCGCCACGACTTCGGCTTCCCT  
TTCGAGGACACCGTTAACTTACCCTCCAGAACGGACTTCCTGGCGGTGCTGAAGCCGAAGGGA

CAGGAAGAATTTCGATGGCAATCTGCAGCAGTTTCAGAAAGAGGACGCCGCATTGACCATCTAT  
GTCCTTCTTAAGCTACCGTTAGACGTCGTCAAAGTCTTCTCCTGCGCGTAACTGGTAGATA

GAGATGCTCCAGAACATCTTTGCTATTTTCAGACAAGATTCC  
CTCTACGAGGTCTTGTAAGAACGATAAAAGTCTGTTCTAAGGAGCT

XhoI

Fig. 5

Chart 2d

0131816

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Chemically synthesized sequence for IFHX402

HinfI

RsaI

ATTCCATTCTCTCGACAACAGACGTACCTGTATGCTGCTCGCTCAGATGAGCCGGATATCCC  
GGTAAGAGAGCTGTTGTCTGCATGGACATACGACGAGCGAGTCTACTCGGCCTATAGGG

EcoRI

CGTCTTCTTGCTGATGGACCGCCACGACTTCGGCTTCCCTCAGGAAGAATTCGATGGCAATC  
GCAGAAGAACGGACTACCTGGCGGTGCTGAAGCCGAAGGGAGTCCTTCTTAAGCTACCGTTAG

HgaI

AGTTTCAGAAAGCACCTGCGATTC  
TCAAAGTCTTTCGTGGACGCTAAGACTGG

Fig. 6

0131816

Chart 2e

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Chemically synthesized sequence for IPHX419

ClbI

CGATAAGCTATGTCTTACAACCTGCTGGGCTTCCATTCTCTGGACAACCGTCGTACTCTGATG  
TATTCGATACAGAATGTTGGACGACCCGAAGGTAAGAGACCTGTTGGCAGCATGAGACTAC

CTGCTCGCTCAGATGAGCCGGATATCCCCGTCTTCTTGCTGATGGACCGCCACGACTTCGGC  
GACGAGCGAGTCTACTCGGCCTATAGGGGCAGAAGAACGGACTACCTGGCGGTGCTGAAGCCG

TTCCCTCAGGAAATCAAACAG  
AAGGGAGTCCTTTAGTTTGTC

PvuII

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Chemically synthesized sequence for IFHX428

Clal

CGATAAGCTATGTCTTACAACCTGCTGGGCTTCCTGCAGCGTTCTTCTAACTTCCAATCTCAG  
TATTCGATACAGAATGTTGGACGACCCGAAGGACGTCGCAAGAAGATTGAAGGTTAGAGTC

AAACTGGCTCAGATGAGCCGGATATCCCCGTCTTCTTGCTGATGGACCGCCACGACTTCGGC  
TTTGACCGAGTCTACTCGGCCTATAGGGGCAGAAGAACGGACTACCTGGCGGTGCTGAAGCCG

TTCCCTCAGGAAATCAAACAG  
AAGGGAGTCCTTTAGTTTGTC

PvuII



Fig. 8

Chart 2g

0131816

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Chemically synthesized sequence for IPHX484

<u>DdeI</u>		<u>HgaI</u>
TGAGGAAGAGTTTGACGGTAATCAGTTC	CAAAAAGCCCCAGCAATCT	
CCTTCTCAA	ACTGCCATTAGTCAAGGTTTTTCGGGGTCGTTAGAACTGG	

## Chart 3a

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IPNX487

IPN- $\beta$ [IPN- $\beta^{9-56}$ →IPN- $\kappa_1^{7-54}$ ]

HinfI



MET	SER	TYR	ASN	LEU	LEU	GLY	PHE	HIS	SER	LEU	ASP	ASN	ARG	ARG
ATG	AGC	TAC	AAC	TTG	CTT	GGA	TTC	CAT	TCT	CTC	GAC	AAC	AGA	CGT
<div style="display: flex; justify-content: space-between;"> <span>5</span> <span>10</span> <span>15</span> </div>														
THR	LEU	MET	LEU	LEU	ALA	GLN	MET	SER	ARG	ILE	SER	PRO	SER	SER
ACC	CTT	ATG	CTG	CTC	GCT	CAG	ATG	AGC	CGG	ATA	TCC	CCG	TCT	TCT
<div style="display: flex; justify-content: space-between;"> <span>20</span> <span>25</span> <span>30</span> </div>														
CYS	LEU	MET	ASP	ARG	HIS	ASP	PHE	GLY	PHE	PRO	GLN	GLU	GLU	PHE
TGC	CTG	ATG	GAC	CGC	CAC	GAC	TTC	GGC	TTC	CCT	CAG	GAA	GAA	TTC
<div style="display: flex; justify-content: space-between;"> <span>35</span> <span>40</span> <span>45</span> </div>														

HgaI



ASP	GLY	ASN	GLN	PHE	GLN	LYS	ALA	PRO	ALA	ILE	LEU	THR	ILE	TYR
GAT	GGC	AAT	CAG	TTT	CAG	AAA	GCA	CCT	GCG	ATT	CTG	ACC	ATC	TAT
<div style="display: flex; justify-content: space-between;"> <span>50</span> <span>55</span> <span>60</span> </div>														
GLU	MET	LEU	GLN	ASN	ILE	PHE	ALA	ILE	PHE	ARG	GLN	ASP	SER	SER
GAG	ATG	CTC	CAG	AAC	ATC	TTT	GCT	ATT	TTC	AGA	CAA	GAT	TCA	TCT
<div style="display: flex; justify-content: space-between;"> <span>65</span> <span>70</span> <span>75</span> </div>														
SER	THR	GLY	TRP	ASN	GLU	THR	ILE	VAL	GLU	ASN	LEU	LEU	ALA	ASN
AGC	ACT	GGC	TGG	AAT	GAG	ACT	ATT	GTT	GAG	AAC	CTC	CTG	GCT	AAT
<div style="display: flex; justify-content: space-between;"> <span>80</span> <span>85</span> <span>90</span> </div>														
VAL	TYR	HIS	GLN	ILE	ASN	HIS	LEU	LYS	THR	VAL	LEU	GLU	GLU	LYS
GTC	TAT	CAT	CAG	ATA	AAC	CAT	CTG	AAG	ACA	GTC	CTG	GAA	GAA	AAA
<div style="display: flex; justify-content: space-between;"> <span>95</span> <span>100</span> <span>105</span> </div>														
LEU	GLU	LYS	GLU	ASP	PHE	THR	ARG	GLY	LYS	LEU	MET	SER	SER	LEU
CTG	GAG	AAA	GAA	GAT	TTC	ACC	AGG	GGA	AAA	CTC	ATG	AGC	AGT	CTG
<div style="display: flex; justify-content: space-between;"> <span>110</span> <span>115</span> <span>120</span> </div>														
HIS	LEU	LYS	ARG	TYR	TYR	GLY	ARG	ILE	LEU	HIS	TYR	LEU	LYS	ALA
CAC	CTG	AAA	AGA	TAT	TAT	GGG	AGG	ATT	CTG	CAT	TAC	CTG	AAG	GCC
<div style="display: flex; justify-content: space-between;"> <span>125</span> <span>130</span> <span>135</span> </div>														
LYS	GLU	TYR	SER	HIS	CYS	ALA	TRP	THR	ILE	VAL	ARG	VAL	GLU	ILE
AAG	GAG	TAC	AGT	CAC	TGT	GCC	TGG	ACC	ATA	GTC	AGA	GTG	GAA	ATC
<div style="display: flex; justify-content: space-between;"> <span>140</span> <span>145</span> <span>150</span> </div>														
LEU	ARG	ASN	PHE	TYR	PHE	ILE	ASN	ARG	LEU	THR	GLY	TYR	LEU	ARG
CTA	AGG	AAC	TTT	TAC	TTC	ATT	AAC	AGA	CTT	ACA	GGT	TAC	CTC	CGA
<div style="display: flex; justify-content: space-between;"> <span>155</span> <span>160</span> <span>165</span> </div>														

ASN \*\*\*  
AAC TGA

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Chart 3a (Cont'd.)

10 20 30 40 50  
MYSNLLGFHS-LDNRRTLMLL-AQMSRISPSS-CLMDRHDFGF-PQEEFDGNQF-  
60 70 80 90 100  
QKAPAILTIY-EMLQNIFAIF-RQSSSTGWN-ETIVENLLAN-VYHQINHLKT-  
110 120 130 140 150  
VLEEKLEKED-PTRGKLMSSL-HLKRYYGRIL-HYLKKEYSH-CAWTIVRVEI-  
160  
LRNFYFINRL-TGYLRN<

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IPN-β[IPN-β<sup>44-56</sup>->IPN-~~α~~<sup>42-54</sup>][Cys<sup>17</sup>->Ser<sup>17</sup>]

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Chart 3b (Cont'd.)

12/31

10 20 30 40 50  
MSYNLLGFLQ-RSSNFQSQKL-LWQLNGRLEY-CLKDRMNFDI-PEEEFDGNQF-  
60 70 80 90 100  
QKAPAILTIY-EMLQNIFAIF-RQDSSSTGWN-ETIVENLLAN-VYHQINHLKT-  
110 120 130 140 150  
VLEEKLEKED-FTRGKLMSSL-HLKRYYGRIL-HYLKAKKEYSH-CAWTIVRVEI-  
160  
LRNFYFINRL-TGYLRN<

Chart 3c

10/21

IPNX489

IPN- $\beta$ [IPN- $\beta$ <sup>42-56</sup>->IPN- $\beta$ <sup>48-54</sup>][Cys<sup>17</sup>->Ser<sup>17</sup>]

				5					10					15
MET	SER	TYR	ASN	LEU	LEU	GLY	PHE	LEU	GLN	ARG	SER	SER	ASN	PHE
ATG	AGC	TAC	AAC	TTG	CTT	GGA	TTC	CTA	CAA	AGA	AGC	AGC	AAT	TTT
				20					25					30
GLN	SER	GLN	LYS	LEU	LEU	TRP	GLN	LEU	ASN	GLY	ARG	LEU	GLU	TYR
CAG	AGT	CAG	AAG	CTC	CTG	TGG	CAA	TTG	AAT	GGG	AGG	CTT	GAA	TAC
				35					40					45
CYS	LEU	LYS	ASP	ARG	MET	ASN	PHE	ASP	ILE	PRO	GLN	GLU	GLU	PHE
TGC	CTC	AAG	GAC	AGG	ATG	AAC	TTT	GAC	ATC	CCT	CAA	GAA	GAG	TTT
				50					55					60
ASP	GLY	ASN	GLN	PHE	GLN	LYS	ALA	PRO	ALA	ILE	LEU	THR	ILE	TYR
GAC	GGT	AAT	CAG	TTC	CAA	AAA	GCC	CCA	GCA	ATC	TTG	ACC	ATC	TAT
				65					70					75
GLU	MET	LEU	GLN	ASN	ILE	PHE	ALA	ILE	PHE	ARG	GLN	ASP	SER	SER
GAG	ATG	CTC	CAG	AAC	ATC	TTT	GCT	ATT	TTC	AGA	CAA	GAT	TCA	TCT
				80					85					90
SER	THR	GLY	TRP	ASN	GLU	THR	ILE	VAL	GLU	ASN	LEU	LEU	ALA	ASN
AGC	ACT	GGC	TGG	AAT	GAG	ACT	ATT	GTT	GAG	AAC	CTC	CTG	GCT	AAT
				95					100					105
VAL	TYR	HIS	GLN	ILE	ASN	HIS	LEU	LYS	THR	VAL	LEU	GLU	GLU	LYS
GTC	TAT	CAT	CAG	ATA	AAC	CAT	CTG	AAG	ACA	GTC	CTG	GAA	GAA	AAA
				110					115					120
LEU	GLU	LYS	GLU	ASP	PHE	THR	ARG	GLY	LYS	LEU	MET	SER	SER	LEU
CTG	GAG	AAA	GAA	GAT	TTC	ACC	AGG	GGA	AAA	CTC	ATG	AGC	AGT	CTG
				125					130					135
HIS	LEU	LYS	ARG	TYR	TYR	GLY	ARG	ILE	LEU	HIS	TYR	LEU	LYS	ALA
CAC	CTG	AAA	AGA	TAT	TAT	GGG	AGG	ATT	CTG	CAT	TAC	CTG	AAG	GCC
				140					145					150
LYS	GLU	TYR	SER	HIS	CYS	ALA	TRP	THR	ILE	VAL	ARG	VAL	GLU	ILE
AAG	GAG	TAC	AGT	CAC	TGT	GCC	TGG	ACC	ATA	GTC	AGA	GTG	GAA	ATC
				155					160					165
LEU	ARG	ASN	PHE	TYR	PHE	ILE	ASN	ARG	LEU	THR	GLY	TYR	LEU	ARG
CTA	AGG	AAC	TTT	TAC	TTC	ATT	AAC	AGA	CTT	ACA	GGT	TAC	CTC	CGA

ASN \*\*\*  
AAC TGA

190

Chart 3c (Cont'd.)

14/31

10 20 30 40 50  
MSYNLLGFLO-RSSNFQSOKL-LWQLNGRLEY-CLKDRMNFDI-PQEEFDGNQF-  
60 70 80 90 100  
QKAPAILTIY-EMLQNIFAIF-RQDSSSTGWN-ETIVENLLAN-VYHQINHLKT-  
110 120 130 140 150  
VLEEKLEKED-FTRGKLMSSL-HLKRYYYGRIL-HYLKAKEYSH-CAWTIVRVEI-  
160  
LRNFYFINRL-TGYLRN<

## Chart 3d

15/31

IFNX410

$$\text{IFN-}\beta[\text{IFN-}\beta^{2-7} \rightarrow \text{IFN-}\alpha_2^{1-5}][\text{IFN-}\beta^{9-56} \rightarrow \text{IFN-}\alpha_1^{7-54}]$$

Clal															
(N <sub>9</sub> )					5					10					15
	MET	CYS	ASP	LEU	PRO	GLN	PHE	HIS	SER	LEU	ASP	ASN	ARG	ARG	THR
	ATG	TGC	GAC	TTA	CCA	CAA	TTC	CAT	TCT	CTC	GAC	AAC	CGT	CGT	ACT
				20					25					30	
	LEU	MET	LEU	LEU	ALA	GLN	MET	SER	ARG	ILE	SER	PRO	SER	SER	CYS
	CTG	ATG	CTG	CTC	GCT	CAG	ATG	AGC	CGG	ATA	TCC	CCG	TCT	TCT	TGC
				35					40					45	
	LEU	MET	ASP	ARG	HIS	ASP	PHE	GLY	PHE	PRO	GLN	GLU	GLU	PHE	ASP
	CTG	ATG	GAC	CGC	CAC	GAC	TTC	GGC	TTC	CCT	CAG	GAA	GAA	TTC	GAT
				50					55					60	
	GLY	ASN	GLN	PHE	GLN	LYS	ALA	PRO	ALA	ILE	LEU	THR	ILE	TYR	GLU
	GGC	AAT	CAG	TTT	CAG	AAA	GCA	CCT	GCG	ATT	CTG	ACC	ATC	TAC	GAA
				65					70					75	
	MET	LEU	GLN	ASN	ILE	PHE	ALA	ILE	PHE	ARG	GLN	ASP	SER	SER	SER
	ATG	CTG	CAA	AAC	ATC	TTC	GCG	ATC	TTC	CGT	CAA	GAC	TCT	TCC	TCT
				80					85					90	
	THR	GLY	TRP	ASN	GLU	THR	ILE	VAL	GLU	ASN	LEU	LEU	ALA	ASN	VAL
	ACT	GGT	TGG	AAC	GAA	ACT	ATC	GTA	GAA	AAC	CTG	CTG	GCA	AAC	GTA
				95					100					105	
	TYR	HIS	GLN	ILE	ASN	HIS	LEU	LYS	THR	VAL	LEU	GLU	GLU	LYS	LEU
	TAC	CAT	CAG	ATC	AAC	CAT	CTG	AAA	ACC	GTG	CTG	GAA	GAG	AAA	CTG
				110					115					120	
	GLU	LYS	GLU	ASP	PHE	THR	ARG	GLY	LYS	LEU	MET	SER	SER	LEU	HIS
	GAA	AAA	GAA	GAC	TTC	ACC	CGC	GGT	AAA	CTG	ATG	AGC	TCC	CTG	CAT
				125					130					135	
	LEU	LYS	ARG	TYR	TYR	GLY	ARG	ILE	LEU	HIS	TYR	LEU	LYS	ALA	LYS
	CTG	AAA	CGC	TAC	TAT	GGT	CGT	ATC	CTG	CAT	TAC	CTG	AAA	GCT	AAA
				140					145					150	
	GLU	TYR	SER	HIS	CYS	ALA	TRP	THR	ILE	VAL	ARG	VAL	GLU	ILE	LEU
	GAA	TAC	TCT	CAC	TGC	GCA	TGG	ACT	ATT	GTA	CGC	GTT	GAA	ATC	CTG
				155					160					165	
	ARG	ASN	PHE	TYR	PHE	ILE	ASN	ARG	LEU	THR	GLY	TYR	LEU	ARG	ASN
	CGT	AAC	TTC	TAC	TTC	ATC	AAC	CGC	CTG	ACT	GGT	TAC	CTG	CGT	AAC

\*\*\*  
TAA



Fig. 14 a

0131816

Chart 3d (Cont'd.)

16/31

10 20 30 40 50  
MCDLPQFHSL-DNRRTLMLLA-QMSRISPSSC-LMDRHDFGFP-QEFPDGNQFQ-  
60 70 80 90 100  
KAPAILTIYE-MLQNIFAIFR-QDSSSTGWNE-TIVENLLANV-YBQINHLKTV-  
110 120 130 140 150  
LEEKLEKEDF-TRGKLMSSLH-LKRYYGRILH-YLKAKEYSHC-AWTIVRVEIL-  
160  
RNFYFINRLT-GYLRN<

## Chart 3e

17/31

## IPN415

IPN- $\beta$ [IPN- $\beta^{28-46}$ →IPN- $\alpha_1^{28-46}$ ][Cys<sup>17</sup>→Ser<sup>17</sup>][Met<sup>31</sup>→Lys<sup>31</sup>]

Clal															
(N <sub>9</sub> )					5					10					15
	MET	SER	TYR	ASN	LEU	LEU	GLY	PHE	LEU	GLN	ARG	SER	SER	ASN	PHE
	ATG	AGC	TAC	AAC	TTG	CTT	GGA	TTC	CTA	CAA	AGA	AGC	AGC	AAT	TTT
				20					25					30	
	GLN	SER	GLN	LYS	LEU	LEU	TRP	GLN	LEU	ASN	GLY	ARG	SER	CYS	LEU
	CAG	TCT	CAG	AAG	CTC	CTG	TGG	CAA	TTG	AAT	GGG	AGG	TCT	TGC	CTG
				35					40					45	
	LYS	ASP	ARG	HIS	ASP	PHE	GLY	PHE	PRO	GLN	GLU	GLU	PHE	ASP	GLY
	AAG	GAC	CGC	CAC	GAC	TTC	GGC	TTC	CCT	CAG	GAA	GAA	TTC	GAT	GGC
				50					55					60	
	ASN	LEU	GLN	GLN	PHE	GLN	LYS	GLU	ASP	ALA	ALA	LEU	THR	ILE	TYR
	AAT	CTG	CAG	CAG	TTT	CAG	AAA	GAG	GAC	GCC	GCA	TTG	ACC	ATC	TAT
				65					70					75	
	GLU	MET	LEU	GLN	ASN	ILE	PHE	ALA	ILE	PHE	ARG	GLN	ASP	SER	SER
	GAG	ATG	CTC	CAG	AAC	ATC	TTT	GCT	ATT	TTC	AGA	CAA	GAT	TCC	TCG
				80					85					90	
	SER	THR	GLY	TRP	ASN	GLU	THR	ILE	VAL	GLU	ASN	LEU	LEU	ALA	ASN
	AGC	ACT	GGC	TGG	AAT	GAG	ACT	ATT	GTT	GAG	AAC	CTC	CTG	GCT	AAT
				95					100					105	
	VAL	TYR	HIS	GLN	ILE	ASN	HIS	LEU	LYS	THR	VAL	LEU	GLU	GLU	LYS
	GTC	TAT	CAT	CAG	ATA	AAC	CAT	CTG	AAG	ACA	GTC	CTG	GAA	GAA	AAA
				110					115					120	
	LEU	GLU	LYS	GLU	ASP	PHE	THR	ARG	GLY	LYS	LEU	MET	SER	SER	LEU
	CTG	GAG	AAA	GAA	GAT	TTC	ACC	AGG	GGA	AAA	CTC	ATG	AGC	AGT	CTG
				125					130					135	
	HIS	LEU	LYS	ARG	TYR	TYR	GLY	ARG	ILE	LEU	HIS	TYR	LEU	LYS	ALA
	CAC	CTG	AAA	AGA	TAT	TAT	GGG	AGG	ATT	CTG	CAT	TAC	CTG	AAG	GCC
				140					145					150	
	LYS	GLU	TYR	SER	HIS	CYS	ALA	TRP	THR	ILE	VAL	ARG	VAL	GLU	ILE
	AAG	GAG	TAC	AGT	CAC	TGT	GCC	TGG	ACC	ATA	GTC	AGA	GTG	GAA	ATC
				155					160					165	
	LEU	ARG	ASN	PHE	TYR	PHE	ILE	ASN	ARG	LEU	THR	GLY	TYR	LEU	ARG
	CTA	AGG	AAC	TTT	TAC	TTC	ATT	AAC	AGA	CTT	ACA	GGT	TAC	CTC	CGA
ASN ***															
AAC TGA															

Chart 3e (Cont'd.)

18/31

10	20	30	40	50
MSYNLLGFLQ	-RSSNFQSQKL	-LWQLNGRSCL	-KDRHDPGFPQ	-EFPDGNLQQF-
60	70	80	90	100
QKEDAALTIY	-EMLQNIFAIF	-RQDSSSTGWN	-ETIVENLLAN	-VYBQINHLKT-
110	120	130	140	150
VLEEKLEKED	-FTRGKLMSSL	-HLKRYYGRIL	-HYLKAKKEYSH	-CAWTIVRVEI-
160				
LRNFYFINRL	-TGYLRN<			

Chart 3f

19/01

IFNX402

IPN- $\beta$  [ $\beta^{9-56} \rightarrow \alpha_1^{7-54}$ ] [Leu<sup>17</sup>  $\rightarrow$  Cys<sup>17</sup>]

															Hinf I ↓																
					5						10						15														
MET	SER	TYR	ASN	LEU	LEU	GLY	PHE	HIS	SER	LEU	ASP	ASN	ARG	ARG																	
ATG	AGC	TAC	AAC	TTG	CTT	GGA	TTC	CAT	TCT	CTC	GAC	AAC	AGA	CGT																	
					20						25						30														
THR	CYS	MET	LEU	LEU	ALA	GLN	MET	SER	ARG	ILE	SER	PRO	SER	SER																	
ACC	TGT	ATG	CTG	CTC	GCT	CAG	ATG	AGC	CGG	ATA	TCC	CCG	TCT	TCT																	
					35						40						45														
CYS	LEU	MET	ASP	ARG	HIS	ASP	PHE	GLY	PHE	PRO	GLN	GLU	GLU	PHE																	
TGC	CTG	ATG	GAC	CGC	CAC	GAC	TTC	GGC	TTC	CCT	CAG	GAA	GAA	TTC																	
					50						55						60														
ASP	GLY	ASN	GLN	PHE	GLN	LYS	ALA	PRO	ALA	ILE	LEU	THR	ILE	TYR																	
GAT	GGC	AAT	CAG	TTT	CAG	AAA	GCA	CCT	GCG	ATT	CTG	ACC	ATC	TAT																	
					65						70						75														
GLU	MET	LEU	GLN	ASN	ILE	PHE	ALA	ILE	PHE	ARG	GLN	ASP	SER	SER																	
GAG	ATG	CTC	CAG	AAC	ATC	TTT	GCT	ATT	TTC	AGA	CAA	GAT	TCA	TCT																	
					80						85						90														
SER	THR	GLY	TRP	ASN	GLU	THR	ILE	VAL	GLU	ASN	LEU	LEU	ALA	ASN																	
AGC	ACT	GGC	TGG	AAT	GAG	ACT	ATT	GTT	GAG	AAC	CTC	CTG	GCT	AAT																	
					95						100						105														
VAL	TYR	HIS	GLN	ILE	ASN	HIS	LEU	LYS	THR	VAL	LEU	GLU	GLU	LYS																	
GTC	TAT	CAT	CAG	ATA	AAC	CAT	CTG	AAG	ACA	GTC	CTG	GAA	GAA	AAA																	
					110						115						120														
LEU	GLU	LYS	GLU	ASP	PHE	THR	ARG	GLY	LYS	LEU	MET	SER	SER	LEU																	
CTG	GAG	AAA	GAA	GAT	TTC	ACC	AGG	GGA	AAA	CTC	ATG	AGC	AGT	CTG																	
					125						130						135														
HIS	LEU	LYS	ARG	TYR	TYR	GLY	ARG	ILE	LEU	HIS	TYR	LEU	LYS	ALA																	
CAC	CTG	AAA	AGA	TAT	TAT	GGG	AGG	ATT	CTG	CAT	TAC	CTG	AAG	GCC																	
					140						145						150														
LYS	GLU	TYR	SER	HIS	CYS	ALA	TRP	THR	ILE	VAL	ARG	VAL	GLU	ILE																	
AAG	GAG	TAC	AGT	CAC	TGT	GCC	TGG	ACC	ATA	GTC	AGA	GTG	GAA	ATC																	
					155						160						165														
LEU	ARG	ASN	PHE	TYR	PHE	ILE	ASN	ARG	LEU	THR	GLY	TYR	LEU	ARG																	
CTA	AGG	AAC	TTT	TAC	TTC	ATT	AAC	AGA	CTT	ACA	GGT	TAC	CTC	CGA																	

ASN \*\*\*  
AAC TGA

Fig. 16 a

Chart 3f

0131816

20/31

10 20 30 40 50  
MSYNLLGFHS-LDNRRTCMLL-AQMSRISPSS-CLMDRHDFGF-PQEEFDGNQF-  
60 70 80 90 100  
QKAPAILTIY-EMLQNIFAIF-RQDSSSTGWN-ETIVENLLAN-VYHQINHLKT-  
110 120 130 140 150  
VLEEKLEKED-FTRGRLMSSL-HLKRYYGRIL-HYLKKEYSH-CAWTIVRVEI-  
160  
LRNFYFINRL-TGYLRN<

21/31

IFN $\alpha$ 419IFN- $\beta$  [IFN- $\beta$ <sup>9-42</sup> → IFN- $\alpha$ <sub>1</sub><sup>7-48</sup>]

↓  
 ClaI  
 (N<sub>9</sub>)

5 10 15  
 MET SER TYR ASN LEU LEU GLY PHE HIS SER LEU ASP ASN ARG ARG  
 CTG CTG GGC TTC CAT TCT CTG GAC AAC CGT CGT

20 25 30  
 THR LEU MET LEU LEU ALA GLN MET SER ARG ILE SER PRO SER SER  
 ACT CTG ATG CTG CTC GCT CAG ATG AGC CGG ATA TCC CCG TCT TCT

35 40 45  
 CYS LEU MET ASP ARG HIS ASP PHE GLY PHE PRO GLN GLU ILE LYS  
 TGC CTG ATG GAC CGC CAC GAC TTC GGC TTC CCT CAG GAA ATC AAA

↓  
 PvuII

50 55 60  
 GLN LEU GLN GLN PHE GLN LYS GLU ASP ALA ALA LEU THR ILE TYR  
 CAG CTG CAA CAG TTC CAA AAA GAA GAT GCA GCG CTG ACT ATC TAC

65 70 75  
 GLU MET LEU GLN ASN ILE PHE ALA ILE PHE ARG GLN ASP SER SER  
 GAA ATG CTG CAA AAC ATC TTC GCG ATC TTC CGT CAA GAC TCT TCC

80 85 90  
 SER THR GLY TRP ASN GLU THR ILE VAL GLU ASN LEU LEU ALA ASN  
 TCT ACT GGT TGG AAC GAA ACT ATC GTA GAA AAC CTG CTG GCA AAC

95 100 105  
 VAL TYR HIS GLN ILE ASN HIS LEU LYS THR VAL LEU GLU GLU LYS  
 GTA TAC CAT CAG ATC AAC CAT CTG AAA ACC GTG CTG GAA GAG AAA

110 115 120  
 LEU GLU LYS GLU ASP PHE THR ARG GLY LYS LEU MET SER SER LEU  
 CTG GAA AAA GAA GAC TTC ACC CGC GGT AAA CTG ATG AGC TCC CTG

125 130 135  
 HIS LEU LYS ARG TYR TYR GLY ARG ILE LEU HIS TYR LEU LYS ALA  
 CAT CTG AAA CGC TAC TAT GGT CGT ATC CTG CAT TAC CTG AAA GCT

140 145 150  
 LYS GLU TYR SER HIS CYS ALA TRP THR ILE VAL ARG VAL GLU ILE  
 AAA GAA TAC TCT CAC TGC GCA TGG ACT ATT GTA CGC GTT GAA ATC

155 160 165  
 LEU ARG ASN PHE TYR PHE ILE ASN ARG LEU THR GLY TYR LEU ARG  
 CTG CGT AAC TTC TAC TTC ATC AAC CGC CTG ACT GGT TAC CTG CGT

ASN \*\*\*  
 AAC TAA

198

10 20 30 40 50  
MSYNLLGPBS-LDNRRTLMLL-AQMSRISPSS-CLMDREDFGP-PQEIKQLQPF-  
60 70 80 90 100  
QKEDAALTIY-EMLQNI FAIP-RQDSSSTGWN-ETIVENLLAN-VYHQINHLKT-  
110 120 130 140 150  
VLEEKLEKED-FTRGKLMSSL-HLKRYYYGRIL-BYLAKEYSB-CAWTIVRVEI-  
160  
LRNFYFIPNRL-TGYLRN<

## Chart 3b

23/31

IPNX428

IPN- $\beta$ [IPN- $\beta$ <sup>21-42</sup>→IPN- $\alpha$ <sub>1</sub><sup>19-48</sup>][Cys<sup>17</sup>→Ser<sup>17</sup>]

Clal

(N<sub>9</sub>)

				5					10					15	
MET	SER	TYR	ASN	LEU	LEU	GLY	PHE	LEU	GLN	ARG	SER	SER	ASN	PHE	
ATG	TCT	TAC	AAC	CTG	CTG	GGC	TTT	CTG	CAG	CGT	TCT	TCT	AAC	TTC	
				20					25					30	
GLN	SER	GLN	LYS	LEU	ALA	GLN	MET	SER	ARG	ILE	SER	PRO	SER	SER	
CAA	TCT	CAG	AAA	CTG	GCT	CAG	ATG	AGC	CGG	ATA	TCC	CCG	TCT	TCT	
				35					40					45	
CYS	LEU	MET	ASP	ARG	HIS	ASP	PHE	GLY	PHE	PRO	GLN	GLU	ILE	LYS	
TGC	CTG	ATG	GAC	CGC	CAC	GAC	TTC	GGC	TTC	CCT	CAG	GAA	ATC	AAA	
				50					55					60	
GLN	LEU	GLN	GLN	PHE	GLN	LYS	GLU	ASP	ALA	ALA	LEU	THR	ILE	TYR	
CAG	CTG	CAA	CAG	TTC	CAA	AAA	GAA	GAT	GCA	GCG	CTG	ACT	ATC	TAC	
				65					70					75	
GLU	MET	LEU	GLN	ASN	ILE	PHE	ALA	ILE	PHE	ARG	GLN	ASP	SER	SER	
GAA	ATG	CTG	CAA	AAC	ATC	TTC	GCG	ATC	TTC	CGT	CAA	GAC	TCT	TCC	
				80					85					90	
SER	THR	GLY	TRP	ASN	GLU	THR	ILE	VAL	GLU	ASN	LEU	LEU	ALA	ASN	
TCT	ACT	GGT	TGG	AAC	GAA	ACT	ATC	GTA	GAA	AAC	CTG	CTG	GCA	AAC	
				95					100					105	
VAL	TYR	HIS	GLN	ILE	ASN	HIS	LEU	LYS	THR	VAL	LEU	GLU	GLU	LYS	
GTA	TAC	CAT	CAG	ATC	AAC	CAT	CTG	AAA	ACC	GTG	CTG	GAA	GAG	AAA	
				110					115					120	
LEU	GLU	LYS	GLU	ASP	PHE	THR	ARG	GLY	LYS	LEU	MET	SER	SER	LEU	
CTG	GAA	AAA	GAA	GAC	TTC	ACC	CGC	GGT	AAA	CTG	ATG	AGC	TCC	CTG	
				125					130					135	
HIS	LEU	LYS	ARG	TYR	TYR	GLY	ARG	ILE	LEU	HIS	TYR	LEU	LYS	ALA	
CAT	CTG	AAA	CGC	TAC	TAT	GGT	CGT	ATC	CTG	CAT	TAC	CTG	AAA	GCT	
				140					145					150	
LYS	GLU	TYR	SER	HIS	CYS	ALA	TRP	THR	ILE	VAL	ARG	VAL	GLU	ILE	
AAA	GAA	TAC	TCT	CAC	TGC	GCA	TGG	ACT	ATT	GTA	CGC	GTT	GAA	ATC	
				155					160					165	
LEU	ARG	ASN	PHE	TYR	PHE	ILE	ASN	ARG	LEU	THR	GLY	TYR	LEU	ARG	
CTG	CGT	AAC	TTC	TAC	TTC	ATC	AAC	CGC	CTG	ACT	GGT	TAC	CTG	CGT	

ASN \*\*\*

AAC TAA

200



24/31

10	20	30	40	50
MSYNLLGFLQ-RSSNFQSQKL-AQMSRISPSS-CLMDRHDFGF-PQEIKQLQQF-				
50	70	80	90	100
QKEDAALTIY-EMLQNIFAIF-RQDSSSTGWN-ETIVENLLAN-VYHQINHLKT-				
110	120	130	140	150
VLEEKLEKED-FTRGKLMSSL-HLKRYYGRIL-HYLKAREYSH-CAWTIVRVEI-				
160				
LRNFYFINRL-TGYLRN<				

Chart 31

IFNX404

25/31

IFN- $\beta$  [IFN- $\beta$ <sup>44-56</sup> → ~~42-54~~]<sub>1</sub>

MET	SER	TYR	ASN	LEU	LEU	GLY	PHE	LEU	GLN	ARG	SER	SER	ASN	PHE
ATG	AGC	TAC	AAC	TTG	CTT	GGA	TTC	CTA	CAA	AGA	AGC	AGC	AAT	TTT
5 10 15														
GLN	CYS	GLN	LYS	LEU	LEU	TRP	GLN	LEU	ASN	GLY	ARG	LEU	GLU	TYR
CAG	TGT	CAG	AAG	CTC	CTG	TGG	CAA	TTG	AAT	GGG	AGG	CTT	GAA	TAC
20 25 30														
CYS	LEU	LYS	ASP	ARG	MET	ASN	PHE	ASP	ILE	PRO	GLU	GLU	GLU	PHE
TGC	CTC	AAG	GAC	AGG	ATG	AAC	TTT	GAC	ATC	CCT	GAG	GAA	GAG	TTT
35 40 45														
ASP	GLY	ASN	GLN	PHE	GLN	LYS	ALA	PRO	ALA	ILE	LEU	THR	ILE	TYR
GAC	GGT	AAT	CAG	TTC	CAA	AAA	GCC	CCA	GCA	ATC	TTG	ACC	ATC	TAT
50 55 60														
GLU	MET	LEU	GLN	ASN	ILE	PHE	ALA	ILE	PHE	ARG	GLN	ASP	SER	SER
GAG	ATG	CTC	CAG	AAC	ATC	TTT	GCT	ATT	TTC	AGA	CAA	GAT	TCA	TCT
65 70 75														
SER	THR	GLY	TRP	ASN	GLU	THR	ILE	VAL	GLU	ASN	LEU	LEU	ALA	ASN
AGC	ACT	GGC	TGG	AAT	GAG	ACT	ATT	GTT	GAG	AAC	CTC	CTG	GCT	AAT
80 85 90														
VAL	TYR	HIS	GLN	ILE	ASN	HIS	LEU	LYS	THR	VAL	LEU	GLU	GLU	LYS
GTC	TAT	CAT	CAG	ATA	AAC	CAT	CTG	AAG	ACA	GTC	CTG	GAA	GAA	AAA
95 100 105														
LEU	GLU	LYS	GLU	ASP	PHE	THR	ARG	GLY	LYS	LEU	MET	SER	SER	LEU
CTG	GAG	AAA	GAA	GAT	TTC	ACC	AGG	GGA	AAA	CTC	ATG	AGC	AGT	CTG
110 115 120														
HIS	LEU	LYS	ARG	TYR	TYR	GLY	ARG	ILE	LEU	HIS	TYR	LEU	LYS	ALA
CAC	CTG	AAA	AGA	TAT	TAT	GGG	AGG	ATT	CTG	CAT	TAC	CTG	AAG	GCC
125 130 135														
LYS	GLU	TYR	SER	HIS	CYS	ALA	TRP	THR	ILE	VAL	ARG	VAL	GLU	ILE
AAG	GAG	TAC	AGT	CAC	TGT	GCC	TGG	ACC	ATA	GTC	AGA	GTG	GAA	ATC
140 145 150														
LEU	ARG	ASN	PHE	TYR	PHE	ILE	ASN	ARG	LEU	THR	GLY	TYR	LEU	ARG
CTA	AGG	AAC	TTT	TAC	TTC	ATT	AAC	AGA	CTT	ACA	GGT	TAC	CTC	CGA
155 160 165														

ASN \*\*\*  
AAC TGA

Fig. 19 a

Chart 31 (Cont'd.)

0131816

26/21

10 20 30 40 50  
MSYNLLGFLQ-RSSNFQCQKL-LWQLNGRLEY-CLKDRMNFDI-PEEFPDGNQF-  
60 70 80 90 100  
QKAPAILTIY-EMLQNIFAIF-RQDSSSTGWN-ETIVENLLAN-VYHQINHLKT-  
110 120 130 140 150  
VLEEKLEKED-FTRGKLMSL-HLKRYYGRIL-HYLKKEYSH-CAWTIVRVEI-  
160  
LRNFYFINRL-TGYLRN<

27/31

Synthetic IPN- $\beta$  gene

Clal

PstI  
10

5  
CGA TAA GCT MET SER TYR ASN LEU LEU GLY PHE LEU GLN  
ATG TCT TAC AAC CTG CTG GGC TTC CTG CAG

15 20  
ARG SER SER ASN PHE GLN CYS GLN LYS LEU LEU TRP GLN  
CGT TCT TCT AAC TTC CAA TGC CAG AAA CTG CTG TGG CAA

XmaIII

25 30 35  
LEU ASN GLY ARG LEU GLU TYR CYS LEU LYS ASP ARG MET  
CTG AAC GGC CGC CTG GAA TAC TGC CTG AAA GAC CGC ATG

PvuII

41 45  
ASN PHE ASP ILE PRO GLU GLU ILE LYS GLN LEU GLN GLN  
AAC TTT GAT ATC CCA GAA GAA ATC AAA CAG CTG CAA CAG

50 55 60  
PHE GLN LYS GLU ASP ALA ALA LEU THR ILE TYR GLU MET  
TTC CAA AAA GAA GAT GCA GCG CTG ACT ATC TAC GAA ATG

NruI

HinfI

65 71 75  
LEU GLN ASN ILE PHE ALA ILE PHE ARG GLN ASP SER SER  
CTG CAA AAC ATC TTC GCG ATC TTC CGT CAA GAC TCT TCC

80 85  
SER THR GLY TRP ASN GLU THR ILE VAL GLU ASN LEU LEU  
TCT ACT GGT TGG AAC GAA ACT ATC GTA GAA AAC CTG CTG

AccI

90 95 100  
ALA ASN VAL TYR HIS GLN ILE ASN HIS LEU LYS THR VAL  
GCA AAC GTA TAC CAT CAG ATC AAC CAT CTG AAA ACC GTG

SacII

105 110  
LEU GLU GLU LYS LEU GLU LYS GLU ASP PHE THR ARG GLY  
CTG GAA GAG AAA CTG GAA AAA GAA GAC TTC ACC CGC GGT

SacI

115 122 125  
LYS LEU MET SER SER LEU HIS LEU LYS ARG TYR TYR GLY  
AAA CTG ATG AGC TCC CTG CAT CTG AAA CGC TAC TAT GGT

130 135 140  
ARG ILE LEU HIS TYR LEU LYS ALA LYS GLU TYR SER HIS  
CGT ATC CTG CAT TAC CTG AAA GCT AAA GAA TAC TCT CAC

Chart 3j (Cont'd.)

20/31

MstI

				145					150				
CYS	ALA	TRP	THR	ILE	VAL	ARG	VAL	GLU	ILE	LEU	ARG	ASN	
TGC	GCA	TGG	ACT	ATT	GTA	CGC	GTT	GAA	ATC	CTG	CGT	AAC	

BstEII

	155					160						166	
PHE	TYR	PHE	ILE	ASN	ARG	LEU	THR	GLY	TYR	LEU	ARG	ASN	
TTC	TAC	TTC	ATC	AAC	CGC	CTG	ACT	GGT	TAC	CTG	CGT	AAC	

BamHI

TER  
TAA GGA TCC

R<AMSYNLLGFLQRSSNFQCQKLLWQLNGRLEYCLKDRMNFDIPEEIKQLQQF  
QKEDAALTIYEMLQNIFAIFRODSSSTGWNETIVENLLANVYHQINHLKTVLE  
EKLEKEDFTRGKLMSSLHLKRYYGRIHLKKEYSHCAWTIVRVEILRNFYF  
INRLTGylRN<GS

29/31

IFNX406

IFN- $\beta$ { $\beta^{1-56} \rightarrow \alpha_2^{1-53}$ }{Leu<sup>16</sup>→Cys<sup>16</sup>}

TRIPLE LETTER CODE  
(\*\*\* REPRESENTS TERMINATOR SEQUENCE)

5	10	15
MET-CYS-ASP-LEU-PRO-GLN-THR-HIS-SER-LEU-GLY-SER-ARG-ARG-THR-		
ATG TGC GAC TTA CCA CAA ACT CAT TCT CTC GGC TCT AGA CGT ACC		
20	25	30
CYS-MET-LEU-LEU-ALA-GLN-MET-ARG-LYS-ILE-SER-LEU-PHE-SER-CYS-		
TGT ATG CTG CTC GCT CAG ATG AGA AAG ATA TCC CTG TTC TCT TGC		
35	40	45
LEU-LYS-ASP-ARG-HIS-ASP-PHE-GLY-PHE-PRO-GLN-GLU-GLU-PHE-GLY-		
CTG AAG GAC CGC CAC GAC TTC GGC TTC CCT CAG GAA GAA TTC GGC		
50	55	60
ASN-GLN-PHE-GLN-LYS-ALA-GLU-THR-ILE-LEU-THR-ILE-TYR-GLU-MET-		
AAT CAG TTT CAG AAA GCT GAA ACG ATT CTG ACC ATC TAT GAG ATG		
65	70	75
LEU-GLN-ASN-ILE-PHE-ALA-ILE-PHE-ARG-GLN-ASP-SER-SER-SER-THR-		
CTC CAG AAC ATC TTT GCT ATT TTC AGA CAA GAT TCA TCT AGC ACT		
80	85	90
GLY-TRP-ASN-GLU-THR-ILE-VAL-GLU-ASN-LEU-LEU-ALA-ASN-VAL-TYR-		
GGC TGG AAT GAG ACT ATT GTT GAG AAC CTC CTG GCT AAT GTC TAT		
95	100	105
HIS-GLN-ILE-ASN-HIS-LEU-LYS-THR-VAL-LEU-GLU-GLU-LYS-LEU-GLU-		
CAT CAG ATA AAC CAT CTG AAG ACA GTC CTG GAA GAA AAA CTG GAG		
110	115	120
LYS-GLU-ASP-PHE-THR-ARG-GLY-LYS-LEU-MET-SER-SER-LEU-HIS-LEU-		
AAA GAA GAT TTC ACC AGG GGA AAA CTC ATG AGC AGT CTG CAC CTG		
125	130	135
LYS-ARG-TYR-TYR-GLY-ARG-ILE-LEU-HIS-TYR-LEU-LYS-ALA-LYS-GLU-		
AAA AGA TAT TAT GGG AGG ATT CTG CAT TAC CTG AAG GCC AAG GAG		
140	145	150
TYR-SER-HIS-CYS-ALA-TRP-THR-ILE-VAL-ARG-VAL-GLU-ILE-LEU-ARG-		
TAC AGT CAC TGT GCC TGG ACC ATA GTC AGA GTG GAA ATC CTA AGG		
155	160	165
ASN-PHE-TYR-PHE-ILE-ASN-ARG-LEU-THR-GLY-TYR-LEU-ARG-ASN-***-		
AAC TTT TAC TTC ATT AAC AGA CTT ACA GGT TAC CTC CGA AAC TGA		

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IFNX403

IFN- $\beta$ { $\beta^{9-56} + \alpha_2^{7-53}$ }{Leu<sup>17</sup>→Cys<sup>17</sup>}

TRIPLE LETTER CODE  
 (\*\*\*) REPRESENTS TERMINATOR SEQUENCE)

5	10	15
MET-SER-TYR-ASN-LEU-LEU-GLY-PHE-HIS-SER-LEU-GLY-SER-ARG-ARG-		
ATG AGC TAC AAC TTG CTT GGA TTC CAT TCT CTC GGC TCT AGA CGT		
20	25	30
THR-CYS-MET-LEU-LEU-ALA-GLN-MET-ARG-LYS-ILE-SER-LEU-PHE-SER-		
ACC TGT ATG CTG CTC GCT CAG ATG AGA AAG ATA TCC CTG TTC TCT		
35	40	45
CYS-LEU-LYS-ASP-ARG-HIS-ASP-PHE-GLY-PHE-PRO-GLN-GLU-GLU-PHE-		
TGC CTG AAG GAC CGC CAC GAC TTC GGC TTC CCT CAG GAA GAA TTC		
50	55	60
GLY-ASN-GLN-PHE-GLN-LYS-ALA-GLU-THR-ILE-LEU-THR-ILE-TYR-GLU-		
GGC AAT CAG TTT CAG AAA GCT GAA ACG ATT CTG ACC ATC TAT GAG		
65	70	75
MET-LEU-GLN-ASN-ILE-PHE-ALA-ILE-PHE-ARG-GLN-ASP-SER-SER-SER-		
ATG CTC CAG AAC ATC TTT GCT ATT TTC AGA CAA GAT TCA TCT AGC		
80	85	90
THR-GLY-TRP-ASN-GLU-THR-ILE-VAL-GLU-ASN-LEU-LEU-ALA-ASN-VAL-		
ACT GGC TGG AAT GAG ACT ATT GTT GAG AAC CTC CTG GCT AAT GTC		
95	100	105
TYR-HIS-GLN-ILE-ASN-HIS-LEU-LYS-THR-VAL-LEU-GLU-GLU-LYS-LEU-		
TAT CAT CAG ATA AAC CAT CTG AAG ACA GTC CTG GAA GAA AAA CTG		
110	115	120
GLU-LYS-GLU-ASP-PHE-THR-ARG-GLY-LYS-LEU-MET-SER-SER-LEU-HIS-		
GAG AAA GAA GAT TTC ACC AGG GGA AAA CTC ATG AGC AGT CTG CAC		
125	130	135
LEU-LYS-ARG-TYR-TYR-GLY-ARG-ILE-LEU-HIS-TYR-LEU-LYS-ALA-LYS-		
CTG AAA AGA TAT TAT GGG AGG ATT CTG CAT TAC CTG AAG GCC AAG		
140	145	150
GLU-TYR-SER-HIS-CYS-ALA-TRP-THR-ILE-VAL-ARG-VAL-GLU-ILE-LEU-		
GAG TAC AGT CAC TGT GCC TGG ACC ATA GTC AGA GTG GAA ATC CTA		
155	160	165
ARG-ASN-PHE-TYR-PHE-ILE-ASN-ARG-LEU-THR-GLY-TYR-LEU-ARG-ASN-		
AGG AAC TTT TAC TTC ATT AAC AGA CTT ACA GGT TAC CTC CGA AAC		

\*\*\*-  
TGA

207

**0131816**

31/31

HpaI  
HincII RsaI

(TaqI)  
ClaI

\*Transcription initiation

13

CTAGTTAACTAGTAGCGCAAGTTCACGTAAAAAGGGTATCGATAAGCT.ATG.AGC.TAC.AAC.TTG.CTT.

-10 S.D.

Met Ser Tyr Asn Leu Leu

N-terminus mature IFN- $\gamma$

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